Docket No. 60390-AZ-PCT-US/JPW/GJG/JBC

Application
for
United States Letters Patent

To all whom it may concern:

Be it known that

Arlindo L. Castelhano, Bryan McKibben and David J. Witter

have invented certain new and useful improvements in

PYRROLO [2,3d] PYRIMIDINE COMPOSITIONS AND THEIR USE

of which the following is a full, clear and exact description.

PYRROLO[2,3d]PYRIMIDINE COMPOSITIONS AND THEIR USE

Background of the Invention

Adenosine is an ubiquitous modulator of numerous physiological activities, particularly within the cardiovascular and nervous systems. The effects of adenosine appear to be mediated by specific cell surface receptor proteins. Adenosine modulates diverse physiological functions including induction of sedation, vasodilation, suppression of cardiac rate and contractility, inhibition of platelet aggregability, stimulation of gluconeogenesis and inhibition of lipolysis. In addition to its effects on adenylate cyclase, adenosine has been shown to open potassium channels, reduce flux through calcium channels, and inhibit or stimulate phosphoinositide turnover through receptor-mediated mechanisms (See for example, C.E. Muller and B. Stein "Adenosine Receptor Antagonists: Structures and Potential Therapeutic Applications," *Current Pharmaceutical Design*, **2**:501 (1996) and C.E. Muller "A_1-Adenosine Receptor Antagonists," *Exp. Opin. Ther. Patents* 7(5):419 (1997)).

Adenosine receptors belong to the superfamily of purine receptors which are currently subdivided into P_1 (adenosine) and P_2 (ATP, ADP, and other nucleotides) receptors. Four receptor subtypes for the nucleoside adenosine have been cloned so far from various species including humans. Two receptor subtypes (A_1 and A_{2a}) exhibit affinity for adenosine in the nanomolar range while two other known subtypes A_{2b} and A_3 are low-affinity receptors, with affinity for adenosine in the low-micromolar range. A_1 and A_3 adenosine receptor activation can lead to an inhibition of adenylate cyclase activity, while A_{2a} and A_{2b} activation causes a stimulation of adenylate cyclase.

A few A_1 antagonists have been developed for the treatment of cognitive disease, renal failure, and cardiac arrhythmias. It has been suggested that A_{2a} antagonists may be beneficial for patients suffering from Morbus Parkinson (Parkinson's disease). Particularly in view of the potential for local delivery, adenosine receptor antagonists may be valuable for treatment of allergic inflammation and asthma. Available information (for example, Nyce & Metzger "DNA antisense Therapy for Asthma in an Animal Model" *Nature* (1997) 385: 721-5)indicates that in this pathophysiologic context, A_1 antagonists may block contraction of smooth muscle

underlying respiratory epithelia, while A_{2b} or A_3 receptor antagonists may block mast cell degranulation, mitigating the release of histamine and other inflammatory mediators. A_{2b} receptors have been discovered throughout the gastrointestinal tract, especially in the colon and the intestinal epithelia. It has been suggested that A_{2b} receptors mediate cAMP response (Strohmeier *et al.*, *J. Bio. Chem.* (1995) 270:2387-94).

Adenosine receptors have also been shown to exist on the retinas of various mammalian species including bovine, porcine, monkey, rat, guinea pig, mouse, rabbit and human (See, Blazynski *et al.*, *Discrete Distributions of Adenosine Receptors in Mammalian Retina, Journal of Neurochemistry,* volume 54, pages 648-655 (1990); Woods *et al.*, *Characterization of Adenosine A_1-Receptor Binding Sites in Bovine Retinal Membranes, Experimental Eye Research,* volume 53, pages 325-331 (1991); and Braas *et al.*, *Endogenous adenosine and adenosine receptors localized to ganglion cells of the retina, Proceedings of the National Academy of Science,* volume 84, pages 3906-3910 (1987)). Recently, Williams reported the observation of adenosine transport sites in a cultured human retinal cell line (Williams *et al.*, *Nucleoside Transport Sites in a Cultured Human Retinal Cell Line Established By SV-40 T Antigen Gene, Current Eye Research,* volume 13, pages 109-118 (1994)).

Compounds which regulate the uptake of adenosine uptake have previously been suggested as potential therapeutic agents for the treatment of retinal and optic nerve head damage. In U.S. Patent No. 5,780,450 to Shade, Shade discusses the use of adenosine uptake inhibitors for treating eye disorders. Shade does not disclose the use of specific A_3 receptor inhibitors. The entire contents of U.S. Patent No. 5,780,450 are hereby incorporated herein by reference.

Additional adenosine receptor antagonists are needed as pharmacological tools and are of considerable interest as drugs for the above-referenced disease states and/or conditions.

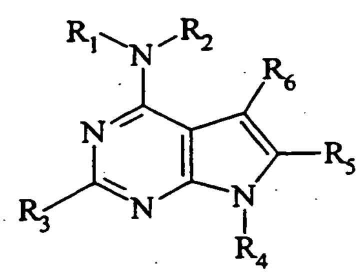
Summary of the Invention

The present invention is based, at least in part, on the discovery that certain N-6 substituted 7-deazapurines, described *infra*, can be used to treat a N-6 substituted 7-

deazapurine responsive state. Examples of such states include those in which the activity of the adenosine receptors is increased, .*e.g.*, bronchitis, gastrointestinal disorders, or asthma. These states can be characterized in that adenosine receptor activation can lead to the inhibition or stimulation of adenylate cyclase activity. Compositions and methods of the invention include enantiomerically or diastereomerically pure N-6 substituted 7-deazapurines. Preferred N-6 substituted 7-deazapurines include those which have an acetamide, carboxamide, substituted cyclohexyl, .*e.g.*, cyclohexanol, or a urea moiety attached to the N-6 nitrogen through an alkylene chain.

The present invention pertains to methods for modulating an adenosine receptor(s) in a mammal by administering to the mammal a therapeutically effective amount of a N-6 substituted 7-deazapurine, such that modulation of the adenosine receptor's activity occurs. Suitable adenosine receptors include the families of A_1, A_2, or A_3. In a preferred embodiment, the N-6 substituted 7-deazapurine is a adenosine receptor antagonist.

The invention further pertains to methods for treating N-6 substituted 7-deazapurine disorders, *e.g.*, asthma, bronchitis, allergic rhinitis, chronic obstructive pulmonary disease, renal disorders, gastrointestinal disorders, and eye disorders, in a mammal by administering to the mammal a therapeutically effective amount of a N-6 substituted 7-deazapurine, such that treatment of the disorder in the mammal occurs. Suitable N-6 substituted 7 deazapurines include those illustrated by the general formula I:

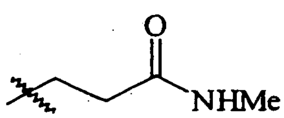


(I)

and pharmaceutically acceptable salts thereof. R_1 and R_2 are each independently a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety or together form a substituted or unsubstituted heterocyclic ring. R_3 is a substituted or

unsubstituted alkyl, aryl, or alkylaryl moiety. R_4 is a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety. R_5 and R_6 are each independently a halogen atom, *e.g.*, chlorine, fluorine, or bromine, a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety or R_4 and R_5 or R_5 and R_6 together form substituted or unsubstituted heterocyclic or carbocyclic ring.

In certain embodiments, R_1 and R_2 can each independently be a substituted or unsubstituted cycloalkyl or heteroarylalkyl moieties. In other embodiments, R_3 is a hydrogen atom or a substituted or unsubstituted heteroaryl moiety. In still other embodiments, R_4, R_5 and R_6 can each be independently a heteroaryl moieties. In a preferred embodiment, R_1 is a hydrogen atom, R_2 is a cyclohexanol, *e.g.*, *trans*-cyclohexanol, R_3 is phenyl, R_4 is a hydrogen atom, R_5 is a methyl group and R_6 is a methyl group. In still another embodiment, R_1 is a hydrogen atom, R_2 is



, R_3 is phenyl, R_4 is a hydrogen atom and R_5 and R_6 are methyl groups.

The invention further pertains to pharmaceutical compositions for treating a N-6 substituted 7-deazapurine responsive state in a mammal, *e.g.*, asthma, bronchitis, allergi rhinitis, chronic obstructive pulmonary disease, renal disorders, gastrointestinal disorders, and eye disorders. The pharmaceutical composition includes a therapeuticall effective amount of a N-6 substituted 7-deazapurine and a pharmaceutically acceptable carrier.

The present invention also pertains to packaged pharmaceutical compositions fo treating a N-6 substituted 7-deazapurine responsive state in a mammal. The packaged pharmaceutical composition includes a container holding a therapeutically effective amount of at least one N-6 substituted 7-deazapurine and instructions for using the N-6 substituted 7-deazapurine for treating a N-6 substituted 7-deazapurine responsive state i a mammal.

The invention further pertains to compounds of formula I wherein:

R_1 is hydrogen;

R_2 is substituted or unsubstituted cycloalkyl, substituted or unsubstituted alkyl, or R_1 and R_2 together form a substituted or unsubstituted heterocyclic ring;

R_3 is unsubstituted or substituted aryl;

R_4 is hydrogen; and

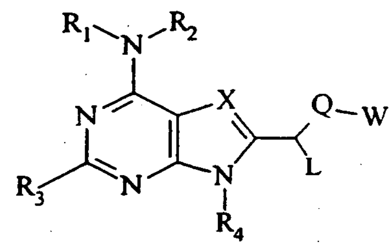
R_5 and R_6 are each independently hydrogen or alkyl, and pharmaceutically acceptable salts thereof. The deazapurines of this embodiment may advantageously be selective A_3 receptor antagonists. These compounds may be useful for numerous therapeutic uses such as, for example, the treatment of asthma, kidney failure associatec with heart failure, and glaucoma. In a particularly preferred embodiment, the deazapurine is a water soluble prodrug that is capable of being metabolized in vivo to a: active drug by, for example, esterase catalyzed hydrolysis.

In yet another embodiment, the invention features a method for inhibiting the activity of an adenosine receptor (*e.g.*, A_3) in a cell, by contacting the cell with N-6 substituted 7-deazapurine (*e.g.*, preferably, an adenosine receptor antagonist).

In another aspect, the invention features a method for treating damage to the ey(of an animal(*e.g.*, a human) by administering to the animal an effective amount of an N 6 substituted 7-deazapurine of formula I. Preferably, the N-6 substituted 7-deazapurine is an antagonist of A_3 adenosine receptors in cells of the animal. The damage is to the retina or the optic nerve head and may be acute or chronic. The damage may be the result of, for example, glaucoma, edema, ischemia, hypoxia or trauma.

The invention also features a pharmaceutical composition comprising a N-6 substituted 7-deazapurine of formula I. Preferably, the pharmaceutical preparation is a: ophthalmic formulation (*e.g.*, an periocular, retrobulbar or intraocular injection formulation, a systemic formulation, or a surgical irrigating solution).

In yet another embodiment, the invention features a deazapurine having the formula II:



(II)

wherein

X is N or CR_6;

R_1 and R_2 are each independently hydrogen, or substituted or unsubstituted alkoxy, aminoalkyl, alkyl, aryl, or alkylaryl, or together form a substituted or unsubstituted heterocyclic ring, provided that both R_1 and R_2 are both not hydrogen;

R_3 is substituted or unsubstituted alkyl, arylalkyl, or aryl;

R_4 is hydrogen or substituted or unsubstituted C_1– C_6 alkyl;

L is hydrogen, substituted or unsubstituted alkyl, or R_4 and L together form a substituted or unsubstituted heterocyclic or carbocyclic ring;

R_6 is hydrogen, substituted or unsubstituted alkyl, or halogen;

Q is CH_2, O, S, or NR_7, wherein R_7 is hydrogen or substituted or unsubstituted C_1– C_6 alkyl; and

W is unsubstituted or substituted alkyl, cycloalkyl, aryl, arylalkyl, biaryl, heteroaryl, substituted carbonyl, substituted thiocarbonyl, or substituted sulfonyl;

provided that if R_3 is pyrrolidino, then R_4 is not methyl. The invention also pertains to pharmaceutically acceptable salts and prodrugs of the compounds of the invention.

In an advantageous embodiment, X is CR_6 and Q is CH_2, O, S, or NH in formula II, wherein R_6 is as defined above.

In another embodiment of formula II, X is N.

The invention further pertains to a method for inhibiting the activity of an adenosine receptor (*e.g.*, an A_{2b} adenosine receptor) in a cell by contacting the cell with a compound of the invention. Preferably, the compound is an antagonist of the receptor.

The invention also pertains to a method for treating a gastrointestinal disorder (*e.g.*, diarrhea) or a respiratory disorder (*e.g.*, allergic rhinitis, chronic obstructive pulmonary disease) in an animal by administering to an animal an effective amount of a compound of formula II (*e.g.*, an antagonist of A_{2b}). Preferably, the animal is a human.

Detailed Description

The features and other details of the invention will now be more particularly described and pointed out in the claims. It will be understood that the particular embodiments of the invention are shown by way of illustration and not as limitations of the invention. The principle features of this invention can be employed in various embodiments without departing from the scope of the invention.

The present invention pertains to methods for treating a N-6 substituted 7-deazapurine responsive state in a mammal. The methods include administration of a therapeutically effective amount of a N-6 substituted 7-deazapurine, described *infra*, to the mammal, such that treatment of the N-6 substituted 7-deazapurine responsive state i the mammal occurs.

The language "N-6 substituted 7-deazapurine responsive state" is intended to include a disease state or condition characterized by its responsiveness to treatment wit a N-6 substituted 7-deazapurine of the invention as described *infra*, *e.g.*, the treatment includes a significant diminishment of at least one symptom or effect of the state achieved with a N-6 substituted 7-deazapurine of the invention. Typically such states are associated with an increase of adenosine within a host such that the host often experiences physiological symptoms which include, but are not limited to, release of toxins, inflammation, coma, water retention, weight gain or weight loss, pancreatitis, emphysema, rheumatoid arthritis, osteoarthritis, multiple organ failure, infant and adul respiratory distress syndrome, allergic rhinitis, chronic obstructive pulmonary disease, eye disorders, gastrointestinal disorders, skin tumor promotion, immunodeficiency and asthma. (See for example, C.E. Muller and B. Stein "Adenosine Receptor Antagonists Structures and Potential Therapeutic Applications," *Current Pharmaceutical Design*, 2:501 (1996) and C.E. Muller "A_1-Adenosine Receptor Antagonists," *Exp. Opin. Ther Patents* 7(5):419 (1997) and I. Feoktistove, R. Polosa, S. T. Holgate and I. Biaggioni

"Adenosine A_{2B} receptors: a novel therapeutic target in asthma?" TiPS **19**; 148 (1998)). The effects often associated with such symptoms include, but are not limited to, fever, shortness of breath, nausea, diarrhea, weakness, headache, and even death. In one embodiment, a N-6 substituted 7-deazapurine responsive state includes those disease states which are mediated by stimulation of adenosine receptors, *e.g.*, A_1, A_{2a}, A_{2b}, A_3 etc., such that calcium concentrations in cells and/or activation of PLC (phospholipase C) is modulated. In a preferred embodiment, a N-6 substituted 7-deazapurine responsive state is associated with adenosine receptor(s), *e.g.*, the N-6 substituted 7-deazapurine acts as an antagonist. Examples of suitable responsive states which can be treated by the compounds of the invention, *e.g.*, adenosine receptor subtypes which mediate biological effects, include central nervous system (CNS) effects, cardiovascular effects, renal effects, respiratory effects, immunological effects, gastro-intestinal effects and metabolic effects. The relative amount of adenosine in a subject can be associated with the effects listed below; that is increased levels of adenosine can trigger an effect, *e.g.*, an undesired physiological response, *e.g.*, an asthmatic attack.

CNS effects include decreased transmitter release (A_1), sedation (A_1), decreased locomotor activity (A_{2a}), anticonvulsant activity, chemoreceptor stimulation (A_2) and hyperalgesia. Therapeutic applications of the inventive compounds include treatment of dementia, Alzheimer's disease and memory enhancement.

Cardiovascular effects include vasodilation (A_{2a}), (A_{2b}) and (A_3), vasoconstriction (A_1), bradycardia (A_1), platelet inhibition (A_{2a}), negative cardiac inotropy and dromotropy (A_1), arrhythmia, tachycardia and angiogenesis. Therapeutic applications of the inventive compounds include, for example, prevention of ischaemia-induced impairment of the heart and cardiotonics, myocardial tissue protection and restoration of cardiac function.

Renal effects include decreased GFR (A_1), mesangial cell contraction (A_1), antidiuresis (A_1) and inhibition of renin release (A_1). Suitable therapeutic applications of the inventive compounds include use of the inventive compounds as diuretic, natriuretic, potassium-sparing, kidney-protective/prevention of acute renal failure, antihypertensive, anti-oedematous and anti-nephritic agents.

Respiratory effects include bronchodilation (A_2), bronchoconstriction (A_1), chronic obstructive pulmonary disease, allergic rhinitis, mucus secretion and respiratory depression (A_2). Suitable therapeutic applications for the compounds of the invention include anti-asthmatic applications, treatment of lung disease after transplantation and respiratory disorders.

Immunological effects include immunosuppression (A_2), neutrophil chemotaxis (A_1), neutrophil superoxide generation (A_{2a}) and mast cell degranulation (A_{2b} and A_3) Therapeutic applications of antagonists include allergic and non allergic inflammation, *e.g.*, release of histamine and other inflammatory mediators.

Gastrointestinal effects include inhibition of acid secretion (A_1) Therapeutic application may include reflux and ulcerative conditions Gastrointestinal effects also include colonic, intestinal and diarrheal disease, *e.g.*, diarrheal disease associated with intestinal inflammation (A_{2b}).

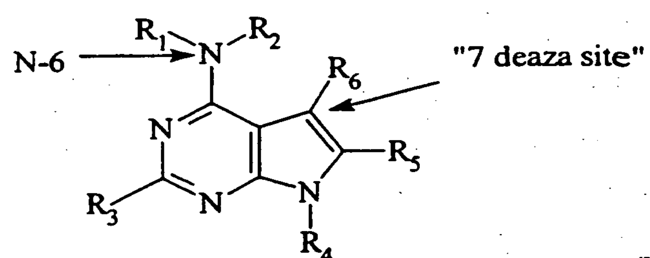
Eye disorders include retinal and optic nerve head injury and trauma related disorders (A_3). In a preferred embodiment, the eye disorder is glaucoma.

Other therapeutic applications of the compounds of the invention include treatment of obesity (lipolytic properties), hypertension, treatment of depression, sedative, anxiolytic, as antileptics and as laxatives, *e.g.*, effecting motility without causing diarrhea.

The term "disease state" is intended to include those conditions caused by or associated with unwanted levels of adenosine, adenylyl cyclase activity, increased physiological activity associated with aberrant stimulation of adenosine receptors and/or an increase in cAMP. In one embodiment, the disease state is, for example, asthma, chronic obstructive pulmonary disease, allergic rhinitis, bronchitis, renal disorders, gastrointestinal disorders, or eye disorders. Additional examples include chronic bronchitis and cystic fibrosis. Suitable examples of inflammatory diseases include non-lymphocytic leukemia, myocardial ischaemia, angina, infarction, cerebrovascular ischaemia, intermittent claudication, critical limb ischemia, venous hypertension, varicose veins, venous ulceration and arteriosclerosis. Impaired reperfusion states include, for example, any post-surgical trauma, such as reconstructive surgery, thrombolysis or angioplasty.

The language "treatment of a N-6 substituted 7-deazapurine responsive state" or "treating a N-6 substituted 7-deazapurine responsive state" is intended to include changes in a disease state or condition, as described above, such that physiological symptoms in a mammal can be significantly diminished or minimized. The language also includes control, prevention or inhibition of physiological symptoms or effects associated with an aberrant amount of adenosine. In one preferred embodiment, the control of the disease state or condition is such that the disease state or condition is eradicated. In another preferred embodiment, the control is selective such that aberrant levels of adenosine receptor activity are controlled while other physiologic systems and parameters are unaffected.

The term "N-6 substituted 7-deazapurine" is art recognized and is intended to include those compounds having the formula **I:**



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"N-substituted 7-deazapurine" includes pharmaceutically acceptable salts thereof, and, in one embodiment, also includes certain N-6 substituted purines described herein.

In certain embodiments, the N-6 substituted 7-deazapurine is not N-6 benzyl or N-6 phenylethyl substituted. In other embodiments, R_4 is not benzyl or phenylethyl substituted. In preferred embodiments, R_1 and R_2 are both not hydrogen atoms. In still other preferred embodiments, R_3 is not a hydrogen atom.

The language "therapeutically effective amount" of an N-6 substituted 7-deazapurine, described *infra*, is that amount of a therapeutic compound necessary or sufficient to perform its intended function within a mammal, *e.g.*, treat a N-6 substituted 7-deazapurine responsive state, or a disease state in a mammal. An effective amount of the therapeutic compound can vary according to factors such as the amount of the causative agent already present in the mammal, the age, sex, and weight of the mammal, and the ability of the therapeutic compounds of the present invention to affect a N-6

substituted 7-deazapurine responsive state in the mammal. One of ordinary skill in the art would be able to study the aforementioned factors and make a determination regarding the effective amount of the therapeutic compound without undue experimentation. An *in vitro* or *in vivo* assay also can be used to determine an "effective amount" of the therapeutic compounds described *infra*. The ordinarily skilled artisan would select an appropriate amount of the therapeutic compound for use in the aforementioned assay or as a therapeutic treatment.

A therapeutically effective amount preferably diminishes at least one symptom or effect associated with the N-6 substituted 7-deazapurine responsive state or condition being treated by at least about 20%, (more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80%) relative to untreated subjects. Assays can be designed by one skilled in the art to measure the diminishment of such symptoms and/or effects. Any art recognized assay capable of measuring such parameters are intended to be included as part of this invention. For example, if asthma is the state being treated, then the volume of air expended from the lungs of a subject can be measured before and after treatment for measurement of increase in the volume using an art recognized technique. Likewise, if inflammation is the state being treated, then the area which is inflamed can be measured before and after treatment for measurement of diminishment in the area inflamed using an art recognized technique.

The term “cell” includes both prokaryotic and eukaryotic cells.

The term “animal” includes any organism with adenosine receptors or any organism susceptible to a N-6-substituted 7-deazapurine responsive state. Examples of animals include yeast, mammals, reptiles, and birds. It also includes transgenic animals.

The term "mammal" is art recognized and is intended to include an animal, more preferably a warm-blooded animal, most preferably cattle, sheep, pigs, horses, dogs, cats, rats, mice, and humans. Mammals susceptible to a N-6 substituted 7-deazapurine responsive state, inflammation, emphysema, asthma, central nervous system conditions, or acute respiratory distress syndrome, for example, are included as part of this invention.

In another aspect, the present invention pertains to methods for modulating an adenosine receptor(s) in a mammal by administering to the mammal a therapeutically effective amount of a N-6 substituted 7-deazapurine, such that modulation of the adenosine receptor in the mammal occurs. Suitable adenosine receptors include the families of A_1, A_2, or A_3. In a preferred embodiment, the N-6 substituted 7-deazapurine is an adenosine receptor antagonist.

The language "modulating an adenosine receptor" is intended to include those instances where a compound interacts with an adenosine receptor(s), causing increased, decreased or abnormal physiological activity associated with an adenosine receptor or subsequent cascade effects resulting from the modulation of the adenosine receptor. Physiological activities associated with adenosine receptors include induction of sedation, vasodilation, suppression of cardiac rate and contractility, inhibition of platelet aggregbility, stimulation of gluconeogenesis, inhibition of lipolysis, opening of potassium channels, reducing flux of calcium channels, etc.

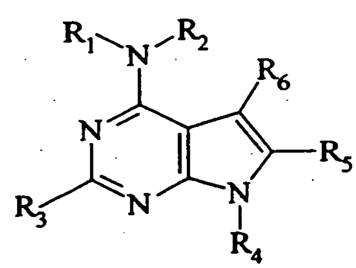
The terms "modulate", "modulating" and "modulation" are intended to include preventing, eradicating, or inhibiting the resulting increase of undesired physiological activity associated with abnormal stimulation of an adenosine receptor, *e.g.*, in the context of the therapeutic methods of the invention. In another embodiment, the term modulate includes antagonistic effects, *e.g.*, diminishment of the activity or production of mediators of allergy and allergic inflammation which results from the overstimulation of adenosine receptor(s). For example, the therapeutic deazapurines of the invention can interact with an adenosine receptor to inhibit, for example, adenylate cyclase activity.

The language "condition characterized by aberrant adenosine receptor activity" is intended to include those diseases, disorders or conditions which are associated with aberrant stimulation of an adenosine receptor, in that the stimulation of the receptor causes a biochemical and or physiological chain of events that is directly or indirectly associated with the disease, disorder or condition. This stimulation of an adenosine receptor does not have to be the sole causative agent of the disease, disorder or condition but merely be responsible for causing some of the symptoms typically associated with the disease, disorder, or condition being treated. The aberrant stimulation of the receptor can be the sole factor or at least one other agent can be involved in the state being

treated. Examples of conditions include those disease states listed *supra*, including inflammation, gastrointestinal disorders and those symptoms manifested by the presence of increased adenosine receptor activity. Preferred examples include those symptoms associated with asthma, allergic rhinitis, chronic obstructive pulmonary disease, emphysema, bronchitis, gastrointestinal disorders and glaucoma.

The language "treating or treatment of a condition characterized by aberrant adenosine receptor activity" is intended to include the alleviation of or diminishment of at least one symptom typically associated with the condition. The treatment also includes alleviation or diminishment of more than one symptom. Preferably, the treatment cures, *e.g.*, substantially eliminates, the symptoms associated with the condition.

The present invention pertains to compounds, N-6 substituted 7-deazapurines, having the formula I:



(I)

wherein

R_1 and R_2 are each independently a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety or together form a substituted or unsubstituted heterocyclic ring;

R_3 is a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety;

R_4 is a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety. R_5 and R_6 are each independently a halogen atom, *e.g.*, chlorine, fluorine, or bromine, a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety or R_4 and R_5 or R_5 and R_6 together form a substituted or unsubstitute heterocyclic or carbocyclic ring. Also included, are pharmaceutically acceptable salts c the N-6 substituted 7-deazapurines.

In certain embodiments, R_1 and R_2 can each independently be a substituted or unsubstituted cycloalkyl or heteroarylalkyl moieties. In other embodiments, R_3 is a hydrogen atom or a substituted or unsubstituted heteroaryl moiety. In still other embodiments, R_4, R_5 and R_6 can each be independently a heteroaryl moiety.

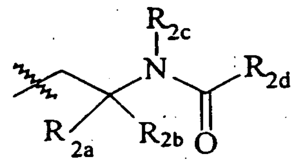
In one embodiment, R_1 is a hydrogen atom, R_2 is a substituted or unsubstituted cyclohexane, cyclopentyl, cyclobutyl or cyclopropane moiety, R_3 is a substituted or unsubstituted phenyl moiety, R_4 is a hydrogen atom and R_5 and R_6 are both methyl groups.

In another embodiment, R_2 is a cyclohexanol, a cyclohexanediol, a cyclohexylsulfonamide, a cyclohexanamide, a cyclohexylester, a cyclohexene, a cyclopentanol or a cyclopentanediol and R_3 is a phenyl moiety.

In still another embodiment, R_1 is a hydrogen atom, R_2 is a cyclohexanol, R_3 is a substituted or unsubstituted phenyl, pyridine, furan, cyclopentane, or thiophene moiety, R_4 is a hydrogen atom, a substituted alkyl, aryl or arylalkyl moiety, and R_5 and R_6 are each independently a hydrogen atom, or a substituted or unsubstituted alkyl, aryl or alkylaryl moiety.

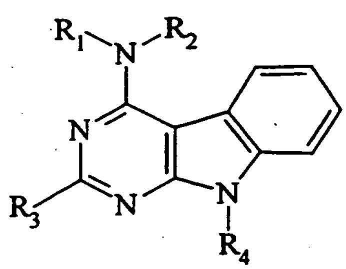
In yet another embodiment, R_1 is a hydrogen atom, R_2 is substituted or unsubstituted alkylamine, arylamine, or alkylarylamine, a substituted or unsubstituted alkylamide, arylamide or alkylarylamide, a substituted or unsubstituted alkylsulfonamide, arylsulfonamide or alkylarylsulfonamide, a substituted or unsubstituted alkylurea, arylurea or alkylarylurea, a substituted or unsubstituted alkylcarbamate, arylcarbamate or alkylarylcarbamate, a substituted or unsubstituted alkylcarboxylic acid, arylcarboxylic acid or alkylarylcarboxylic acid, R_3 is a substituted or unsubstituted phenyl moiety, R_4 is a hydrogen atom and R_5 and R_6 are methyl groups.

In still another embodiment, R_2 is guanidine, a modified guanidine, cyanoguanidine, a thiourea, a thioamide or an amidine.

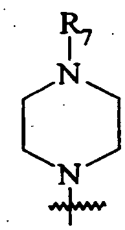
In one embodiment, R_2 can be , wherein R_{2a}-R_{2c} are each independently a hydrogen atom or a saturated or unsaturated alkyl, aryl or alkylaryl moiety and R_{2d} is a hydrogen atom or a saturated or unsaturated alkyl, aryl, or alkylaryl moiety, $NR_{2e}R_{2f}$, or OR_{2g}, wherein R_{2e}-R_{2g} are each independently a hydrogen atom or a saturated or unsaturated alkyl, aryl or alkylaryl moieties. Alternatively, R_{2a} and R_{2b} together can form a carbocyclic or heterocyclic ring having a ring size between about 3 and 8 members, *e.g.*, cyclopropyl, cyclopentyl, cyclohexyl groups.

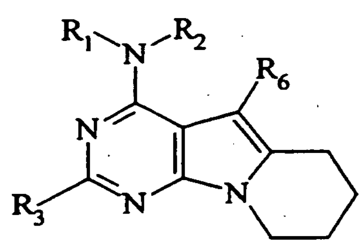
In one aspect of the invention, both R_5 and R_6 are not methyl groups, preferably one of R_5 and R_6 is an alkyl group, *e.g.*, a methyl group, and the other is a hydrogen atom.

In another aspect of the invention, when R_4 is 1-phenylethyl and R_1 is a hydrogen atom, then R_3 is not phenyl, 2-chlorophenyl, 3-chlorophenyl, 4-chlorophenyl, 3,4-dichlorophenyl, 3-methoxyphenyl or 4-methoxyphenyl or when R_4 and R_1 are 1-phenylethyl, then R_3 is not a hydrogen atom or when R_4 is a hydrogen atom and R_3 is a phenyl, then R_1 is not phenylethyl.

In another aspect of the invention, when R_5 and R_6 together form a carbocyclic ring, *e.g.*, , pyrimido[4,5-6]indole, then R_3 is not phenyl when R_4 is 1-(4-methylphenyl)ethyl, phenylisopropyl, phenyl or 1-phenylethyl or when R_3 is not a hydrogen atom when R_4 is 1-phenylethyl. The carbocyclic ring formed by R_5 and R_6 can be either aromatic or aliphatic and can have between 4 and 12 carbon atoms, *e.g.*, naphthyl, phenylcyclohexyl, etc., preferably between 5 and 7 carbon atoms *e.g.*, cyclopentyl or cyclohexyl. Alternatively, R_5 and R_6 together can form a heterocyclic ring, such as those disclosed below. Typical heterocyclic rings include

between 4 and 12 carbon atoms, preferably between 5 and 7 carbon atoms, and can be either aromatic or aliphatic. The heterocyclic ring can be further substituted, including substitution of one or more carbon atoms of the ring structure with one or more heteroatoms.

In still another aspect of the invention, R_1 and R_2 form a heterocyclic ring. Representative examples include, but are not limited to, those heterocyclic rings listed below, such as morpholino, piperazine and the like, *e.g.*, 4-hydroxypiperidines, 4-aminopiperidines. Where R_1 and R_2 together form a piperazino group,  , R_7 can be a hydrogen atom or a substituted or unsubstituted alkyl, aryl or alkylaryl moiety.

In yet another aspect of the invention R_4 and R_5 together can form a heterocycli ring, *e.g.*,  . The heterocyclic ring can be either aromatic or aliphatic and can form a ring having between 4 and 12 carbon atoms, *e.g.*, naphthyl, phenylcyclohexyl, etc. and can be either aromatic or aliphatic, *e.g.*, cyclohexyl, cyclopentyl. The heterocyclic ring can be further substituted, including substitution of carbon atoms of the ring structure with one or more heteroatoms. Alternatively, R_4 anc R_5 together can form a heterocyclic ring, such as those disclosed below.

In certain embodiments, the N-6 substituted 7-deazapurine is not N-6 benzyl or N-6 phenylethyl substituted. In other embodiments, R_4 is not benzyl or phenylethyl substituted. In preferred embodiments, R_1 and R_2 are both not hydrogen atoms. In stil other preferred embodiments, R_3 is not a hydrogen atom.

The compounds of the invention may comprise water-soluble prodrugs which are metabolized *in vivo* to an active drug, *e.g.*, by esterase catalyzed hydrolysis. Examples of potential prodrugs include deazapurines with, for example, R_2 as cycloalkyl substituted with $-OC(O)(Z)NH_2$, wherein Z is a side chain of a naturally or unnaturally occurring amino acid, or analog thereof, an α, β, γ, or ω amino acids, or a dipeptide. Preferred amino acid side chains include those of glycine, alanine, valine, leucine, isoleucine, lysine, α-methylalanine, aminocyclopropane carboxylic acid, azetidine-2-carboxylic acid, β-alanine, γ–aminobutyric acid, alanine-alanine, or glycine-alanine.

In a further embodiment, the invention features deazapurines of the formula (I), wherein:

R_1 is hydrogen;

R_2 is substituted or unsubstituted cycloalkyl, substituted or unsubstituted alkyl, or R_1 and R_2 together form a substituted or unsubstituted heterocyclic ring;

R_3 is unsubstituted or substituted aryl;

R_4 is hydrogen; and

R_5 and R_6 are each independently hydrogen or alkyl,

and pharmaceutically acceptable salts thereof. The deazapurines of this embodiment may potentially be selective A_3 receptor antagonists.

In one embodiment, R_2 is substituted (*e.g.*, hydroxy substituted) or unsubstituted cycloalkyl. In an advantageous subembodiment, R_1 and R_4 are hydrogen, R_3 is unsubstituted or substituted phenyl, and R_5 and R_6 are each alkyl. Preferably R_2 is mono-hydroxycyclopentyl or mono-hydroxycyclohexyl. R_2 also may be substituted with -NH-C(=O)E, wherein E is substituted or unsubstituted C_1-C_4 alkyl (*e.g.*, alkylamine, *e.g.*, ethylamine.).

R_1 and R_2 may also together form a substituted or unsubstituted heterocyclic ring, which may be substituted with an amine or acetamido group.

In another aspect, R_2 may be –A-NHC(=O)B, wherein A is unsubstituted C_1-C_4 alkyl (*e.g.*, ethyl, propyl, butyl), and B is substituted or unsubstituted C_1-C_4 alkyl (*e.g.*, methyl, aminoalkyl, e.g., aminomethyl or aminoethyl, alkylamino, e.g., methylamino, ethylamino), preferably when R_1 and R_4 are hydrogen, R_3 is unsubstituted or

substituted phenyl, and R_5 and R_6 are each alkyl. B may be substituted or unsubstitute cycloalkyl, e.g., cyclopropyl or 1-amino-cyclopropyl.

In another embodiment, R_3 may be substituted or unsubstituted phenyl, preferably when R_5 and R_6 are each alkyl. Preferably, R_3 may have one or more substituents (e.g., *o*-, *m*- or *p*- chlorophenyl, *o*-, *m*- or *p*- fluorophenyl).

Advantageously, R_3 may be substituted or unsubstituted heteroaryl, preferably when R_5 and R_6 are each alkyl. Examples of heteroaryl groups include pyridyl, pyrimidyl, pyridazinyl, pyrazinyl, pyrrolyl, triazolyl, thioazolyl, oxazolyl, oxadiazolyl, furanyl, methylenedioxyphenyl and thiophenyl. Preferably, R_3 is 2-pyridyl, 3-pyridyl, pyridyl, 2-pyrimidyl or 3- pyrimidyl.

Preferably in one embodiment, R_5 and R_6 are each hydrogen. In another, R_5 and R_6 are each methyl.

In a particularly preferred embodiment, the deazapurines of the invention are water-soluble prodrugs that can be metabolized *in vivo* to an active drug, e.g. by estera: catalyzed hydrolysis. Preferably the prodrug comprises an R_2 group which is cycloalk substituted with -OC(O)(Z)NH_2, wherein Z is a side chain of a naturally or unnaturally occurring amino acid, an analog thereof, an α, β, γ, or ω amino acid, or a dipeptide. Examples of preferred side chains include the side chains of glycine, alanine, valine, leucine, isoleucine, lysine, α-methylalanine, aminocyclopropane carboxylic acid, azetidine-2-carboxylic acid, β-alanine, γ–aminobutyric acid, alanine-alanine, or glycin alanine.

In a particularly preferred embodiment, Z is a side chain of glycine, R_2 is cyclohexyl, R_3 is phenyl, and R_5 and R_6 are methyl.

In another embodiment, the deazapurine is 4-(*cis*-3-hydroxycyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine.

In another embodiment, the deazapurine is 4-(*cis*-3-(2-aminoacetoxy) cyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine trifluoroacetic acid salt.

In another embodiment, the deazapurine is 4-(3-acetamido)piperidinyl-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In another embodiment, the deazapurine is 4-(2-N'-methylureapropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In another embodiment, the deazapurine is 4-(2-acetamidobutyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In another embodiment, the deazapurine is 4-(2-N'-methylureabutyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In another embodiment, the deazapurine is 4-(2-aminocyclopropylacetamidoethyl)amino-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In another embodiment, the deazapurine is 4-(*trans*-4-hydroxycyclohexyl)amino-2-(3-chlorophenyl)-7*H*-pyrrolo[2,3d]pyrimidine.

In another embodiment, the deazapurine is 4-(*trans*-4-hydroxycyclohexyl)amino-2-(3-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidine.

In another embodiment, the deazapurine is 4-(*trans*-4-hydroxycyclohexyl)amino-2-(4-pyridyl)-7*H*-pyrrolo[2,3d]pyrimidine.

In yet another embodiment, the invention features a method for inhibiting the activity of an adenosine receptor (*e.g.*, A_1, A_{2A}, A_{2B}, or, preferably, A_3) in a cell, by contacting the cell with N-6 substituted 7-deazapurine (*e.g.*, preferably, an adenosine receptor antagonist).

In another aspect, the invention features a method for treating damage to the eye of an animal(*e.g.*, a human) by administering to the animal an effective amount of an N-6 substituted 7-deazapurine. Preferably, the N-6 substituted 7-deazapurine is an antagonist of A_3 adenosine receptors in cells of the animal. The damage is to the retina or the optic nerve head and may be acute or chronic. The damage may be the result of, for example, glaucoma, edema, ischemia, hypoxia or trauma

In a preferred embodiment, the invention features a deazapurine having the formula II, *supra*, wherein

X is N or CR_6;

R_1 and R_2 are each independently hydrogen, or substituted or unsubstituted alkoxy, aminoalkyl, alkyl, aryl, or alkylaryl, or together form a substituted or unsubstituted heterocyclic ring, provided that both R_1 and R_2 are both not hydrogen;

R_3 is substituted or unsubstituted alkyl, arylalkyl, or aryl;

R_4 is hydrogen or substituted or unsubstituted C_1– C_6 alkyl;

L is hydrogen, substituted or unsubstituted alkyl, or R_4 and L together form a substituted or unsubstituted heterocyclic or carbocyclic ring;

R_6 is hydrogen, substituted or unsubstituted alkyl, or halogen;

Q is CH_2, O, S, or NR_7, wherein R_7 is hydrogen or substituted or unsubstituted C_1– C_6 alkyl; and

W is unsubstituted or substituted alkyl, cycloalkyl, alkynyl, aryl, arylalkyl, biaryl, heteroaryl, substituted carbonyl, substituted thiocarbonyl, or substitute sulfonyl, provided that if R_3 is pyrrolidino, then R_4 is not methyl.

In one embodiment, in compounds of formula II, X is CR_6 and Q is CH_2, O, S, NH. In another embodiment, X is N.

In a further embodiment of compounds of formula II, W is substituted or unsubstituted aryl, 5- or 6- member heteroaryl, or biaryl. W may be substituted with o or more substituents. Examples of substituents include: halogen, hydroxy, alkoxy, amino, aminoalkyl, aminocarboxyamide, CN, CF_3, CO_2R_8, $CONHR_8$, $CONR_8R_9$, SOR SO_2R_8, and $SO_2NR_8R_9$, wherein R_8 and R_9 are each independently hydrogen, or substituted or unsubstituted alkyl, cycloalkyl, aryl, or arylalkyl. Preferably, W may be substituted or unsubstituted phenyl, *e.g.*, methylenedioxyphenyl. W also may be a substituted or unsubstituted 5-membered heteroaryl ring, *e.g.*, pyrrole, pyrazole, oxazol imidazole, triazole, tetrazole, furan, thiophene, thiazole, and oxadiazole. Preferably, W may be a 6-member heteroaryl ring, *e.g.*, pyridyl, pyrimidyl, pyridazinyl, pyrazinal, an thiophenyl. In a preferred embodiment, W is 2-pyridyl, 3- pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, or 5-pyrimidyl.

In one advantageous embodiment of compounds of formula II, Q is NH and W a 3-pyrazolo ring which is unsubstituted or N-substituted by substituted or unsubstitute alkyl, cycloalkyl, aryl, or arylalkyl.

In another embodiment of compounds of formula II, Q is oxygen, and W is a 2-thiazolo ring which is unsubstituted or substituted by substituted or unsubstituted alkyl cycloalkyl, aryl, or arylalkyl.

In another embodiment of compounds of formula II, W is substituted or unsubstituted alkyl, cycloalkyl *e.g.*, cyclopentyl, or arylalkyl. Examples of substituents include halogen, hydroxy , substituted or unsubstituted alkyl, cycloalkyl, aryl, arylalkyl, or NHR_{10}, wherein R_{10} is hydrogen, or substituted or unsubstituted alkyl, cycloalkyl, aryl, or arylalkyl.

In yet another embodiment, the invention features a deazapurine of formula II wherein W is $-(CH_2)_a$-C(=O)Y or $-(CH_2)_a$-C(=S)Y, and a is an integer from 0 to 3, Y is aryl, alkyl, arylalkyl, cycloalkyl, heteroaryl, alkynyl, $NHR_{11}R_{12}$, or, provided that Q is NH, OR_{13}, wherein R_{11}, R_{12} and R_{13} are each independently hydrogen, or unsubstituted or substituted alkyl, aryl, arylalkyl, or cycloalkyl. Preferably, Y is a 5- or 6- member heteroaryl ring.

Furthermore, W may be $-(CH_2)_b-S(=O)_jY$, wherein j is 1 or 2, b is 0, 1, 2, or 3, Y is aryl, alkyl, arylalkyl, cycloalkyl, alkynyl, heteroaryl, $NHR_{14}R_{15}$, provided that whe b is 1, Q is CH_2, , and wherein R_{14}, R_{15}, and R_{16} are each independently hydrogen, or unsubstituted or substituted alkyl, aryl, arylalkyl, or cycloalkyl.

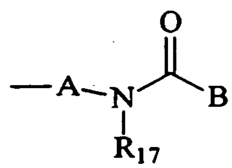
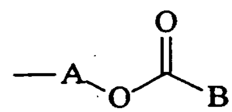
In another embodiment, R_3 is selected from the group consisting of substituted and unsubstituted phenyl, pyridyl, pyrimidyl, pyridazinyl, pyrazinal, pyrrolyl, triazolyl, thioazolyl, oxazolyl, oxadiazolyl, pyrazolyl, furanyl, methylenedioxyphenyl, and thiophenyl. When R_3 is phenyl, it may be substituted with, for example, hydroxyl, alkoxy (*e.g.*, methoxy), alkyl (*e.g.*, tolyl), and halogen,(*e.g.*, *o*-, *m*-, or *p*- fluorophenyl o *o*-, *m*-, or *p*- chlorophenyl). Advantageously, R_3 may be 2-, 3-, or 4- pyridyl or 2- or 3- pyrimidyl.

The invention also pertains to a deazapurine wherein R_6 is hydrogen or C_1-C_3 alkyl. Preferably, R_6 is hydrogen.

The invention also includes deazapurines wherein R_1 is hydrogen, and R_2 is substituted or unsubstituted alkyl or alkoxy, substituted or unsubstituted alkylamine, arylamine, or alkylarylamine, substituted or unsubstituted aminoalkyl, amino aryl, or aminoalkylaryl, substituted or unsubstituted alkylamide, arylamide or alkylarylamide, substituted or unsubstituted alkylsulfonamide, arylsulfonamide or alkylarylsulfonamic substituted or unsubstituted alkylurea, arylurea or alkylarylurea, substituted or

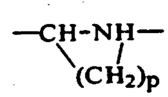
unsubstituted alkylcarbamate, arylcarbamate or alkylarylcarbamate, or substituted or unsubstituted alkylcarboxylic acid, arylcarboxylic acid or alkylarylcarboxylic acid. Preferably, R_2 is substituted or unsubstituted cycloalkyl, *e.g.*, mono- or dihydroxy-substituted cyclohexyl or cyclopentyl (preferably, monohydroxy-substituted cyclohexyl or monohydroxy-substituted cyclopentyl).

Advantageously, R_2 may be of the following formula:

 or 

wherein A is C_1-C_6 alkyl, C_3-C_7 cycloalkyl, a chain of one to seven atoms, or a ring of three to seven atoms, optionally substituted with C_1-C_6 alkyl, halogens, hydroxyl, carboxyl, thiol, or amino groups;

B is methyl, $N(Me)_2$, $N(Et)_2$, NHMe, NHEt, $(CH_2)_rNH_3+$, $NH(CH_2)_rCH_3$, $(CH_2)_rNH_2$, $(CH_2)_rCHCH_3NH_2$, $(CH_2)_rNHMe$, $(CH_2)_rOH$, CH_2CN, $(CH_2)_mCO_2H$, $CHR_{18}R_{19}$, or CHMeOH, wherein r is an integer from 0 to 2, m is 1 or 2, R_{18} is alkyl, R_{19} is NH_3+ or CO_2H or R_{18} and R_{19} together are:



wherein p is 2 or 3; and

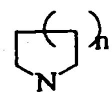
R_{17} is C_1-C_6 alkyl, C_3-C_7 cycloalkyl, a chain of one to seven atoms, or a ring of three to seven atoms, optionally substituted with C_1-C_6 alkyl, halogens, hydroxyl, carboxyl, thiol, or amino groups.

Advantageously, A is unsubstituted or substituted C_1-C_6 alkyl. B may be unsubstituted or unsubstituted C_1-C_6 alkyl.

In a preferred embodiment, R_2 is of the formula –A-NHC(=O)B. In a particularly advantageous embodiment, A is $-CH_2CH_2-$ and B is methyl.

The compounds of the invention may comprise water-soluble prodrugs which ar metabolized *in vivo* to an active drug, *e.g.*, by esterase catalyzed hydrolysis. Examples of potential prodrugs include deazapurines with, for example, R_2 as cycloalkyl substituted with -OC(O)(Z)NH_2, wherein Z is a side chain of a naturally or unnaturally occurring amino acid, or analog thereof, an α, β, γ, or ω amino acid, or a dipeptide. Preferred amino acid side chains include those of glycine, alanine, valine, leucine, isoleucine, lysine, α-methylalanine, aminocyclopropane carboxylic acid, azetidine-2-carboxylic acid, β-alanine, γ–aminobutyric acid, alanine-alanine, or glycine-alanine.

In another embodiment, R_1 and R_2 together are:



wherein n is 1 or 2, and wherein the ring may be optionally substituted with one or more hydroxyl, amino, thiol, carboxyl, halogen, CH_2OH, $CH_2NHC(=O)$alkyl, or $CH_2NHC(=O)NH$alkyl groups. Preferably, n is 1 or 2 and said ring is substituted with -NHC(=O)alkyl.

In one advantageous embodiment, R_1 is hydrogen, R_2 is substituted or unsubstituted C_1-C_6 alkyl, R_3 is substituted or unsubstituted phenyl, R_4 is hydrogen, L hydrogen or substituted or unsubstituted C_1-C_6 alkyl, Q is O, S or NR_7, wherein R_7 is hydrogen or substituted or unsubstituted C_1– C_6 alkyl, and W is substituted or unsubstituted aryl. Preferably, R_2 is –A-NHC(=O)B, wherein A and B are each independently unsubstituted or substituted C_1-C_4 alkyl. For example, A may be CH_2CH_2. B may be, for example, alkyl (*e.g.*, methyl), or aminoalkyl (*e.g.*, aminomethyl). Preferably, R_3 is unsubstituted phenyl and L is hydrogen. R_6 may be methyl or preferably, hydrogen. Preferably, Q is O, S, or NR_7 wherein R_7 is hydrogen or substituted or unsubstituted C_1– C_6 alkyl, *e.g.*, methyl. W is unsubstituted or substituted phenyl (*e.g.*, alkoxy, halogen substituted). Preferably, W is *p*-fluorophenyl *p*- chlorophenyl, or *p*-methoxyphenyl. W may also be heteroaryl, *e.g.*, 2-pyridyl.

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-phenoxymethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-(4-fluorophenoxy)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-(4-chlorophenoxy)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-(4-methoxyphenoxy)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-(2-pyridyloxy)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidin

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-(N-phenylamino)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-(N-methyl-N-phenylamino)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In a particularly preferred embodiment, the deazapurine is 4-(2-N'-methylureaethyl) amino-6-phenoxymethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

The invention further pertains to a method for inhibiting the activity of an adenosine receptor (*e.g.*, an A_{2b} adenosine receptor) in a cell by contacting the cell with compound of the invention. Preferably, the compound is an antagonist of the receptor.

The invention also pertains to a method for treating a gastrointestinal disorder (*e.g.*, diarrhea) in an animal by administering to an animal an effective amount of a compound of the invention (*e.g.*, an antagonist of A_{2b}). Preferably, the animal is a human.

In another embodiment, the invention relates to a pharmaceutical composition containing an N-6 substituted 7-deazapurine of the invention and a pharmaceutically acceptable carrier.

The invention also pertains to a method for treating a N-6 substituted 7-deazapurine responsive state in an animal, by administering to a mammal a therapeutically effective amount of a deazapurine of the invention, such that treatment of a N-6 substituted 7-deazapurine responsive state in the animal occurs. Advantageously, the disease state may be a disorder mediated by adenosine. Examples of preferred disease states include: central nervous system disorders, cardiovascular disorders, renal disorders, inflammatory disorders, allergic disorders, gastrointestinal disorders, eye disorders, and respiratory disorders.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. The term alkyl further includes alkyl groups, which can further include oxygen, nitrogen, sulfur or phosphorous atoms replacing one or more carbons of the hydrocarbon backbone, *e.g.*, oxygen, nitrogen, sulfur or phosphorous atoms. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (*e.g.*, C_1-C_{30} for straight chain, C_3-C_{30} for branched chain), and more preferably 20 or fewer. Likewise, preferred cycloalkyls have from 4-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure.

Moreover, the term alkyl as used throughout the specification and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if

appropriate. Cycloalkyls can be further substituted, *e.g.*, with the substituents described above. An "alkylaryl" moiety is an alkyl substituted with an aryl (*e.g.*, phenylmethyl (benzyl)). The term "alkyl" also includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The term "aryl" as used herein, refers to the radical of aryl groups, including 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, benzoxazole, benzothiazole, triazole, tetrazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles", "heteroaryls" or "heteroaromatics". The aromatic ring can be substituted at one or more ring positions with such substituents as described above, as for example, halogen, hydroxyl, alkoxy, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (*e.g.*, tetralin).

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively. For example, the invention contemplates cyano and propargyl groups.

Unless the number of carbons is otherwise specified, "lower alkyl" as used here means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure, even more preferably

one to three carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths.

The terms "alkoxyalkyl", "polyaminoalkyl" and "thioalkoxyalkyl" refer to alkyl groups, as described above, which further include oxygen, nitrogen or sulfur atoms replacing one or more carbons of the hydrocarbon backbone, *e.g.*, oxygen, nitrogen or sulfur atoms.

The terms "polycyclyl" or "polycyclic radical" refer to the radical of two or more cyclic rings (*e.g.*, cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, *e.g.*, the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkyl, alkylaryl, or an aromatic or heteroaromatic moiety.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorus.

The term “amino acids” includes naturally and unnaturally occurring amino acids found in proteins such as glycine, alanine, valine, cysteine, leucine, isoleucine, serine, threonine, methionine, glutamic acid, aspartic acid, glutamine, asparagine, lysine, arginine, proline, histidine, phenylalanine, tyrosine, and tryptophan. Amino acid analogs include amino acids with lengthened or shortened side chains or variant side chains with appropriate functional groups. Amino acids also include D and L stereoisomers of an amino acid when the structure of the amino acid admits of stereoisomeric forms. The term “dipeptide” includes two or more amino acids linked together. Preferably, dipeptides are two amino acids linked via a peptide linkage.

Particularly preferred dipeptides include, for example, alanine-alanine and glycine-alanine.

It will be noted that the structure of some of the compounds of this invention includes asymmetric carbon atoms. It is to be understood accordingly that the isomers arising from such asymmetry (*e.g.*, all enantiomers and diastereomers) are included within the scope of this invention, unless indicated otherwise. Such isomers can be obtained in substantially pure form by classical separation techniques and by stereochemically controlled synthesis.

The invention further pertains to pharmaceutical compositions for treating a N-substituted 7-deazapurine responsive state in a mammal, *e.g.*, respiratory disorders (*e.g* asthma, bronchitis, chronic obstructive pulmonary disorder, and allergic rhinitis), rena disorders, gastrointestinal disorders, and eye disorders. The pharmaceutical compositi includes a therapeutically effective amount of a N-6 substituted 7-deazapurine, descril *supra*, and a pharmaceutically acceptable carrier. It is to be understood, that all of the deazapurines described above are included for therapeutic treatment. It is to be furthe understood that the deazapurines of the invention can be used alone or in combination with other deazapurines of the invention or in combination with additional therapeutic compounds, such as antibiotics, antiinflammatories, or anticancer agents, for example

The term "antibiotic" is art recognized and is intended to include those substances produced by growing microorganisms and synthetic derivatives thereof, which eliminate or inhibit growth of pathogens and are selectively toxic to the pathog while producing minimal or no deleterious effects upon the infected host subject. Suitable examples of antibiotics include, but are not limited to, the principle classes o aminoglycosides, cephalosporins, chloramphenicols, fuscidic acids, macrolides, penicillins, polymixins, tetracyclines and streptomycins.

The term "antiinflammatory" is art recognized and is intended to include those agents which act on body mechanisms, without directly antagonizing the causative ag of the inflammation such as glucocorticoids, aspirin, ibuprofen, NSAIDS, etc.

The term "anticancer agent" is art recognized and is intended to include those agents which diminish, eradicate, or prevent growth of cancer cells without, preferably, adversely affecting other physiological functions. Representative examples include cisplatin and cyclophosphamide.

When the compounds of the present invention are administered as pharmaceuticals, to humans and mammals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a compound(s) of the present invention within or to the subject such that it can performs its intended function. Typically, such compounds are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

As set out above, certain embodiments of the present compounds can contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable acids. The term "pharmaceutically acceptable salts" in this respect, refers to the relatively non-toxic,

inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, *e.g.*, Berge *et al.* (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19).

In other cases, the compounds of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of compounds of the present invention. These salts can likewise be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form with a suitable base, suc as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondar or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

The term "pharmaceutically acceptable esters" refers to the relatively non-toxic, esterified products of the compounds of the present invention. These esters can be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form or hydroxyl with a suitable esterifying agent. Carboxylic acids can be converted into esters *via* treatment with an alcohol in the presence of a catalyst. Hydroxyl containing derivatives can be converted into esters *via* treatment with an esterifying agent such as alkanoyl halides. The term is further intended to include lower hydrocarbon groups capable of being

solvated under physiological conditions, *e.g.*, alkyl esters, methyl, ethyl and propyl esters. (See, for example, Berge *et al.*, *supra.*)

The invention further contemplates the use of prodrugs which are converted *in vivo* to the therapeutic compounds of the invention (see, *e.g.*, R.B. Silverman, 1992, "The Organic Chemistry of Drug Design and Drug Action", Academic Press, Chp. 8). Such prodrugs can be used to alter the biodistribution (*e.g.*, to allow compounds which would not typically enter the reactive site of the protease) or the pharmacokinetics of the therapeutic compound. For example, a carboxylic acid group, can be esterified, *e.g.*, with a methyl group or an ethyl group to yield an ester. When the ester is administered to a subject, the ester is cleaved, enzymatically or non-enzymatically, reductively or hydrolytically, to reveal the anionic group. An anionic group can be esterified with moieties (*e.g.*, acyloxymethyl esters) which are cleaved to reveal an intermediate compound which subsequently decomposes to yield the active compound. In another embodiment, the prodrug is a reduced form of a sulfate or sulfonate, *e.g.*, a thiol, which is oxidized *in vivo* to the therapeutic compound. Furthermore, an anionic moiety can be esterified to a group which is actively transported *in vivo*, or which is selectively taken up by target organs. The ester can be selected to allow specific targeting of the therapeutic moieties to particular reactive sites, as described below for carrier moieties.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present invention include those suitable for oral, nasal, topical, transdermal, buccal, sublingual, rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium

carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; absorbents, such as kaolin and bentonite clay; lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert dilutents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert dilutents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with

a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active compound in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention. Preferably, the pharmaceutical preparation is an ophthalmic formulation (e.g., an periocular, retrobulbar or intraocular injection formulation, a systemic formulation, or a surgical irrigating solution).

The ophthalmic formulations of the present invention may include one or more deazapurines and a pharmaceutically acceptable vehicle. Various types of vehicles may be used. The vehicles will generally be aqueous in nature. Aqueous solutions are generally preferred, based on case of formulation, as well as a patient's ability to easily administer such compositions by means of instilling one to two drops of the solutions in the affected eyes. However, the deazapurines of the present invention may also be readily incorporated into other types of compositions, such as suspensions, viscous or semi-viscous gels or other types of solid or semi-solid compositions. The ophthalmic

compositions of the present invention may also include various other ingredients, such as buffers, preservatives, co-solvents and viscosity building agents.

An appropriate buffer system (e.g., sodium phosphate, sodium acetate or sodium borate) may be added to prevent pH drift under storage conditions.

Ophthalmic products are typically packaged in multidose form. Preservatives are thus required to prevent microbial contamination during use. Suitable preservatives include: benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, polyquaternium-1, or other agents known to those skilled in the art. Such preservatives are typically employed at a level of from 0.001 to 1.0% weight/volume ("% w/v").

When the deazapurines of the present invention are administered during intraocular surgical procedures, such as through retrobulbar or periocular injection and intraocular perfusion or injection, the use of balanced salt irrigating solutions as vehicles are most preferred. BSS® Sterile Irrigating Solution and BSS Plus® Sterile Intraocular Irrigating Solution (Alcon Laboratories, Inc., Fort Worth, Tex. USA) are examples of physiologically balanced intraocular irrigating solutions. The latter type of solution is described in U.S. Pat. No. 4,550,022 (Garabedian, *et al.*), the entire contents of which are hereby incorporated in the present specification by reference. Retrobulbar and periocular injections are known to those skilled in the art and are described in numerous publications including, for example, *Ophthalmic Surgery: Principles of Practice,* Ed., G. L. Spaeth. W. B. Sanders Co., Philadelphia, Pa., U.S.A., pages 85-87 (1990).

As indicated above, use of deazapurines to prevent or reduce damage to retinal and optic nerve head tissues at the cellular level is a particularly important aspect of one embodiment of the invention. Ophthalmic conditions which may be treated include, but are not limited to, retinopathies, macular degeneration, ocular ischemia, glaucoma, and damage associated with injuries to ophthalmic tissues, such as ischemia reperfusion injuries, photochemical injuries, and injuries associated with ocular surgery, particularly injuries to the retina or optic nerve head by exposure to light or surgical instruments. The compounds may also be used as an adjunct to ophthalmic surgery, such as by vitrea or subconjunctival injection following ophthalmic surgery. The compounds may be used for acute treatment of temporary conditions, or may be administered chronically,

especially in the case of degenerative disease. The compounds may also be used prophylactically, especially prior to ocular surgery or noninvasive ophthalmic procedures, or other types of surgery.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more compounds of the invention in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotoni with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case o dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifunga agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may al: be desirable to include isotonic agents, such as sugars, sodium chloride, and the like in the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical composition of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determin and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that require in order to achieve the desired therapeutic effect and gradually increase the dosage unti the desired effect is achieved.

In general, a suitable daily dose of a compound of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, intravenous and subcutaneous doses of the compounds of this invention for patient, when used for the indicated analgesic effects, will range from about 0.0001 to about 200 mg per kilogram of body weight per day, more preferably from about 0.01 t about 150 mg per kg per day, and still more preferably from about 0.2 to about 140 mg per kg per day.

Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given by forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion o inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administratio is preferred.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systematically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the centra nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

These compounds may be administered to humans and other animals for therap by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powder ointments or drops, including buccally and sublingually.

If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical composition.

The present invention also pertains to packaged pharmaceutical compositions foı treating a N-6 substituted 7 deazapurine responsive state, *e.g.*, undesirable increased adenosine receptor activity in a mammal. The packaged pharmaceutical compositions include a container holding a therapeutically effective amount of at least one deazapurine as described *supra* and instructions for using the deazapurine for treating the deazapurine responsive state in the mammal.

The deazapurines of the invention can be prepared using standard methods for organic synthesis. Deazapurines can be purified by reverse phase HPLC, chromatography, recrystallization, etc. and their structures confirmed by mass spectral analysis, elemental analysis, IR and/or NMR spectroscopy.

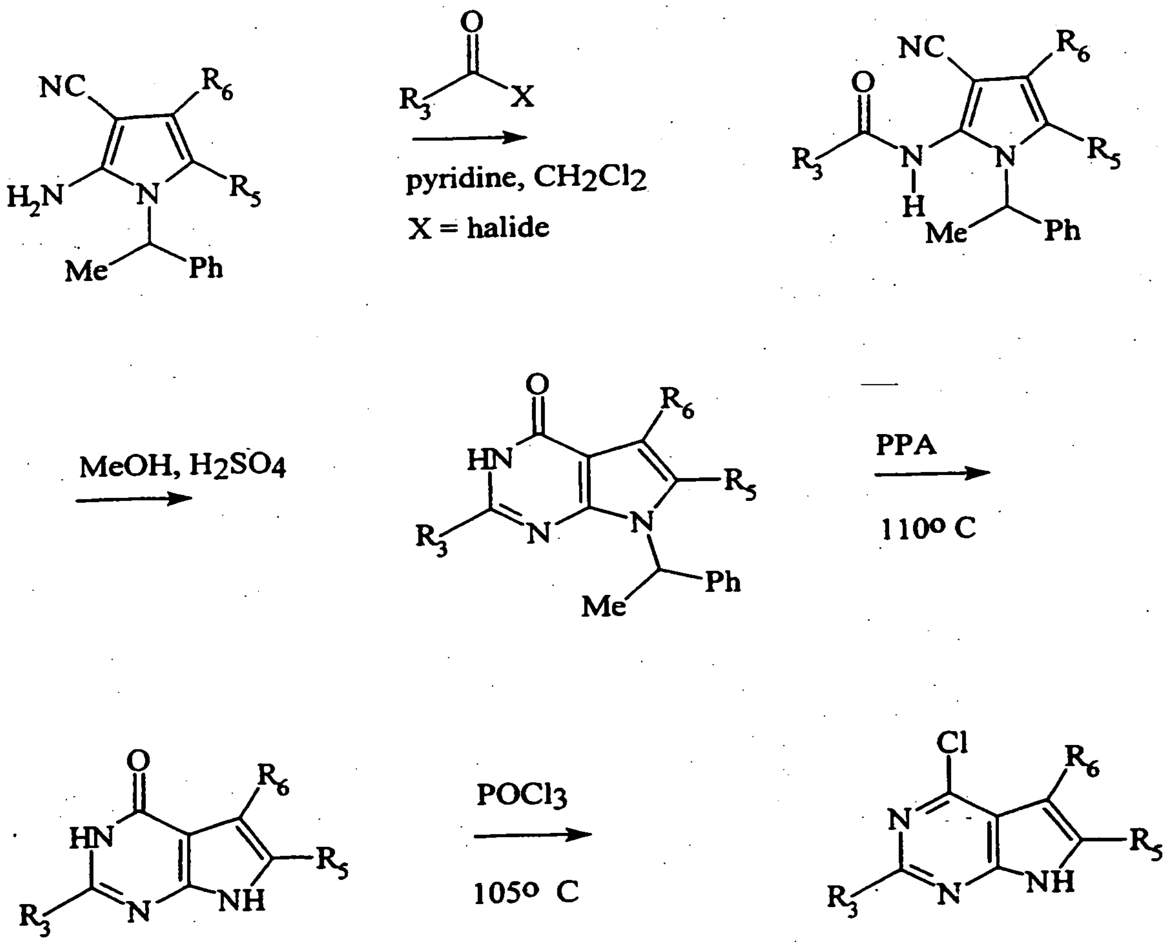
Typically, synthesis of the intermediates as well as the deazapurines of the invention is performed in solution. The addition and removal of one or more protecting group is also typical practice and is known to those skilled in the art. Typical synthetic schemes for the preparation of deazapurine intermediates of the invention are outlined below in Scheme I.

The invention is further illustrated by the following examples which in no way should be construed as being further limiting. The contents of all references, pending patent applications and published patent applications, cited throughout this application, including those referenced in the background section, are hereby incorporated by reference. It should be understood that the models used throughout the examples are accepted models and that the demonstration of efficacy in these models is predictive of efficacy in humans.

The deazapurines of the invention can be prepared using standard methods for organic synthesis. Deazapurines can be purified by reverse phase HPLC, chromatography, recrystallization, etc. and their structures confirmed by mass spectral analysis, elemental analysis, IR and/or NMR spectroscopy.

Typically, synthesis of the intermediates as well as the deazapurines of the invention is performed in solution. The addition and removal of one or more protecting group is also typical practice and is known to those skilled in the art. Typical synthetic schemes for the preparation of deazapurine intermediates of the invention are outlined below in Scheme I.

Scheme I



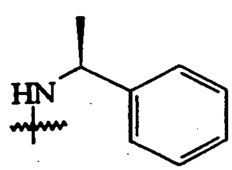
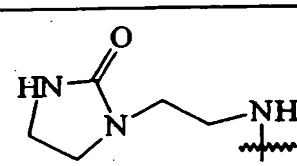
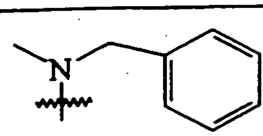
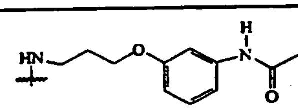
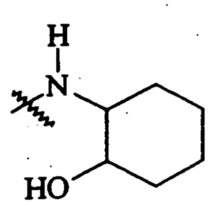
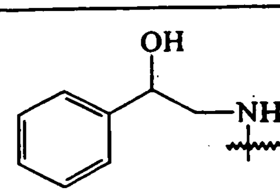
wherein R_3, R_5 and R_6 are as defined above.

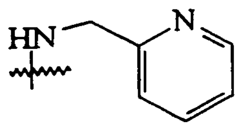
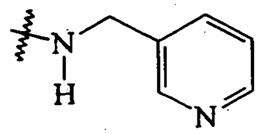
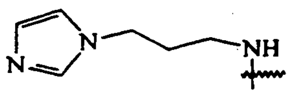
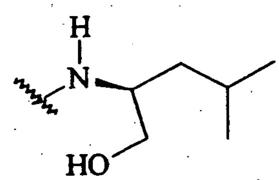
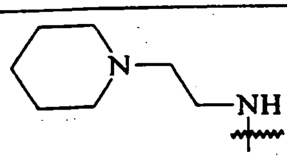
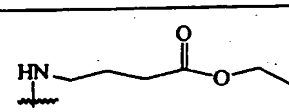
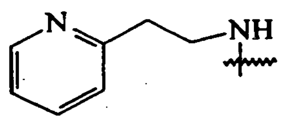
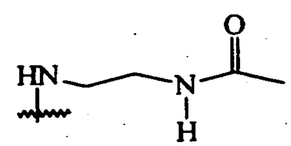
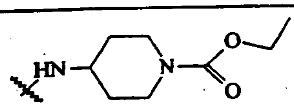
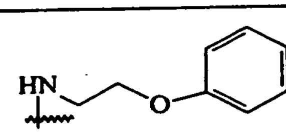
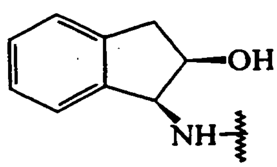
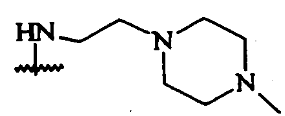
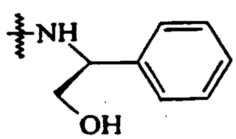
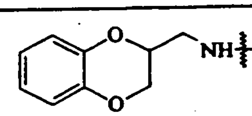
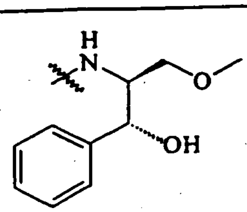
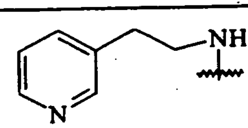
In general, a protected 2-amino-3-cyano-pyrrole can be treated with an acyl halide to form a carboxyamido-3-cyano-pyrrole which can be treated with acidic methanol to effect ring closure to a pyrrolo[2,3d]pyrimidine-4(3*H*)-one (Muller, C.E. *et al.* J. Med. Chem. 40:4396 (1997)). Removal of the pyrrolo protecting group followed

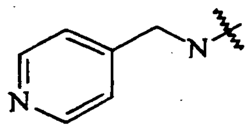
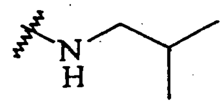
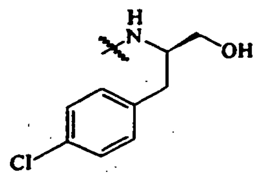
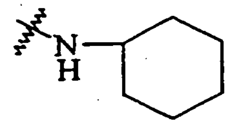
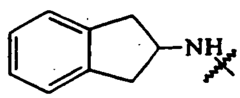
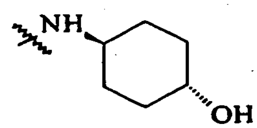
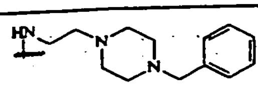
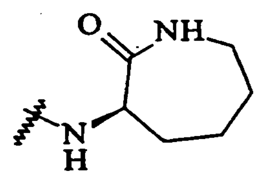
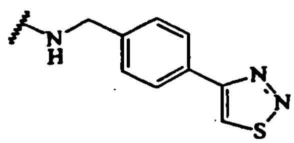
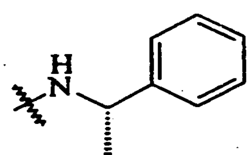
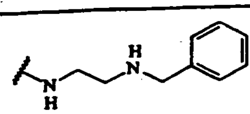
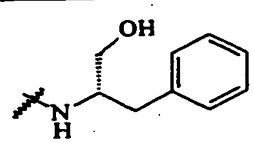
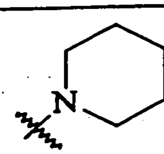
by treatment with a chlorinating reagent, *e.g.*, phosphorous oxychloride, produced substituted or unsubstituted 4-chloro-7H-pyrrolo[2,3d]pyrimidines. Treatment of the chloropyrimidine with amines afforded 7-deazapurines.

For example, as shown in Scheme I, a N-(1-*dl*-phenylethyl)-2-amino-3-cyano-pyrrole was treated with an acyl halide in pyridine and dichloromethane. The resultant N-(1-*dl*-phenylethyl)-2-phenylcarboxyamido-3-cyano-pyrrole was treated with a 10:1 mixture of methanol/sulfuric acid to effect ring closure, resulting in a *dl*-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidine-4(3*H*)-one. Removal of the phenylethyl group by treatment of the pyrimidine with polyphosphoric acid (PPA) followed by $POCl_3$ afforded a key intermediate, the 4-chloro-7H-pyrrolo[2,3d]pyrimidine. Further treatment of the 4-chloro-7H-pyrrolo[2,3d]pyrimidine with various amines listed in Table 1 gives compounds of formula (I) and (II).

TABLE 1

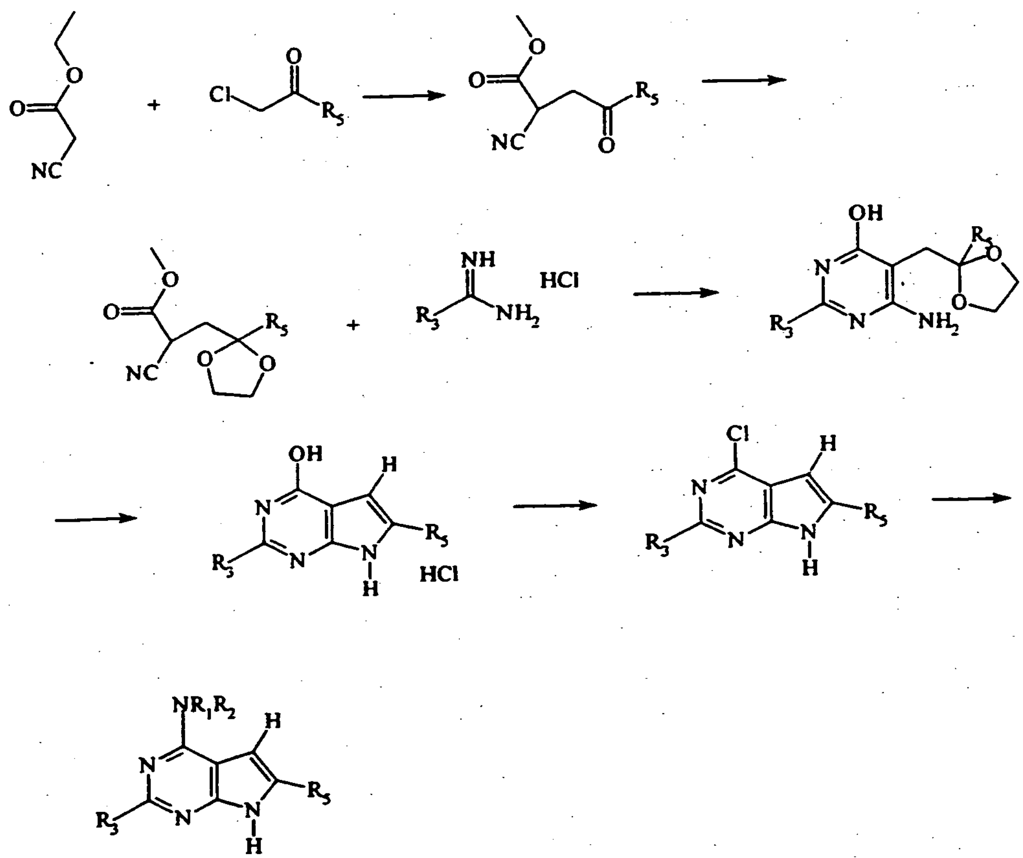
R	$M^+ + H$	R	$M^+ + H$
	343.2		351.27
	343.18		430.35
	337.21		359.44

	330.18		330.45
	347.22		339.47
	350.28		353.41
	344.19		324.45
	394.16		359.38
	371.12		379.40
	359.39		387.41
	403.33		344.48

	330.37		295.2
	407.23		321.2
	355.45		337.53
	441.33		350.2
	413.24		343.2
	372.48		373.2
			307.2

A general approach to prepare 6-substituted pyrroles is depicted in the following scheme (Scheme II).

Scheme II

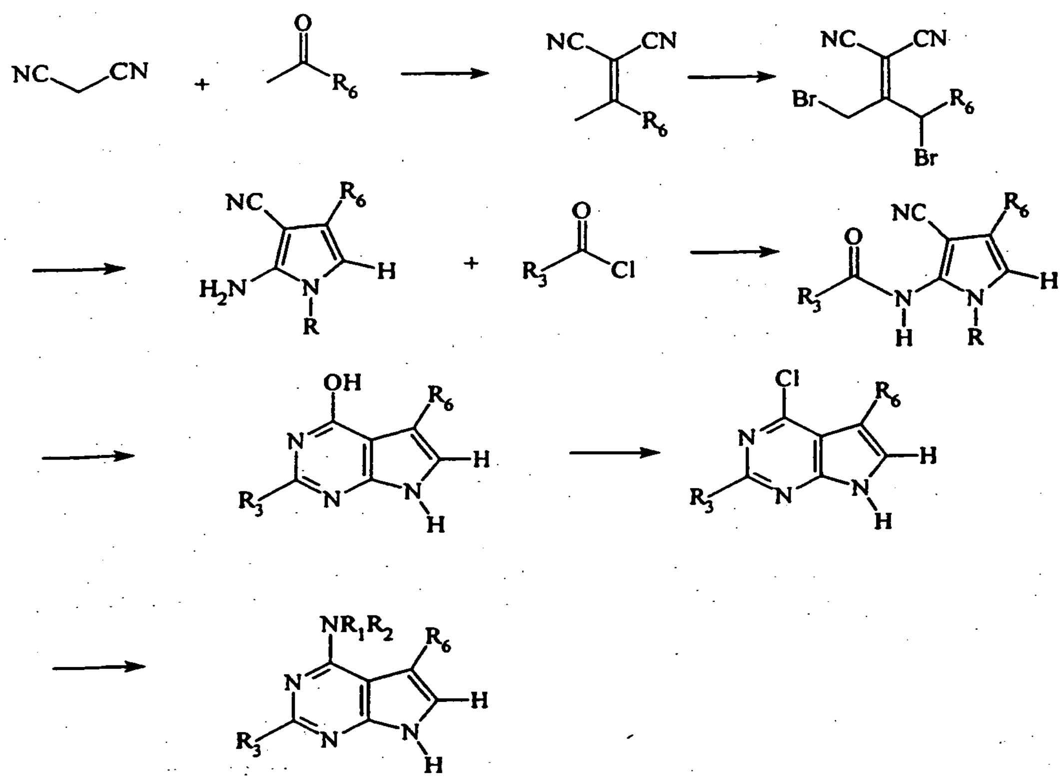


wherein R_1 through R_5 are as defined above.

Transesterification and alkylation of ethyl cyanoacetate with an α-haloketone affords a ketomethylester. Protection of the ketone followed by treatment with an amidine (*e.g.*, alkyl, aryl or alkylaryl) hydrochloride produced the resultant ketal protected pyrimidine. Removal of the protecting group, followed by cyclization and treatment with phosphorous oxychloride afforded the chloride intermediate which coul(be further treated with an amine to afford an amine 6-substituted pyrrole. Additionally, alkylation of the pyrrole nitrogen can be achieved under art recognized conditions.

A general approach to prepare 5-substituted pyrroles is depicted in the followin(scheme (Scheme III).

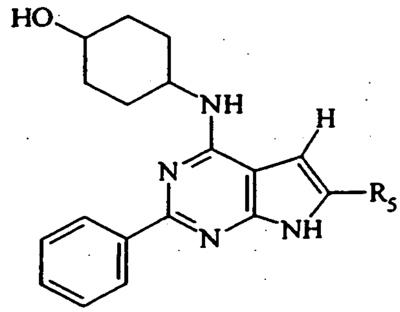
Scheme III



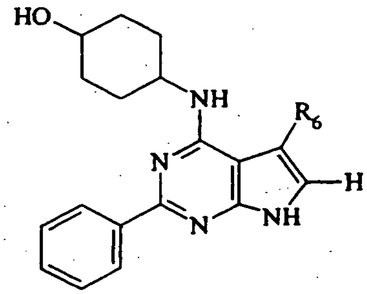
wherein R_1 through R_6 are defined as above and R is a removable protecting group.

Condensation of malononitrile and an excess of a ketone followed by bromination of the product afforded a mixture of starting material, monobrominated and dibrominated products which were treated with an alkylamine, arylamine or alkylarylamine. The resultant amine product was acylated with an acid chloride and the monacylated pyrrole was cyclized in the presence of acid to afford the corresponding pyrimidine. The pyrrole protecting group was removed with polyphosphoric acid and treated with phosphorous oxychloride to produce a chlorinated product. The chlorinated pyrrole could subsequently be treated with an amine to produce an amino 5-substituted pyrrole. Alkylation of the pyrrole nitrogen can be achieved under art recognized conditions.

Schemes IV and V depict methods for preparing the deazapurines 1 and 2 of the invention.



1



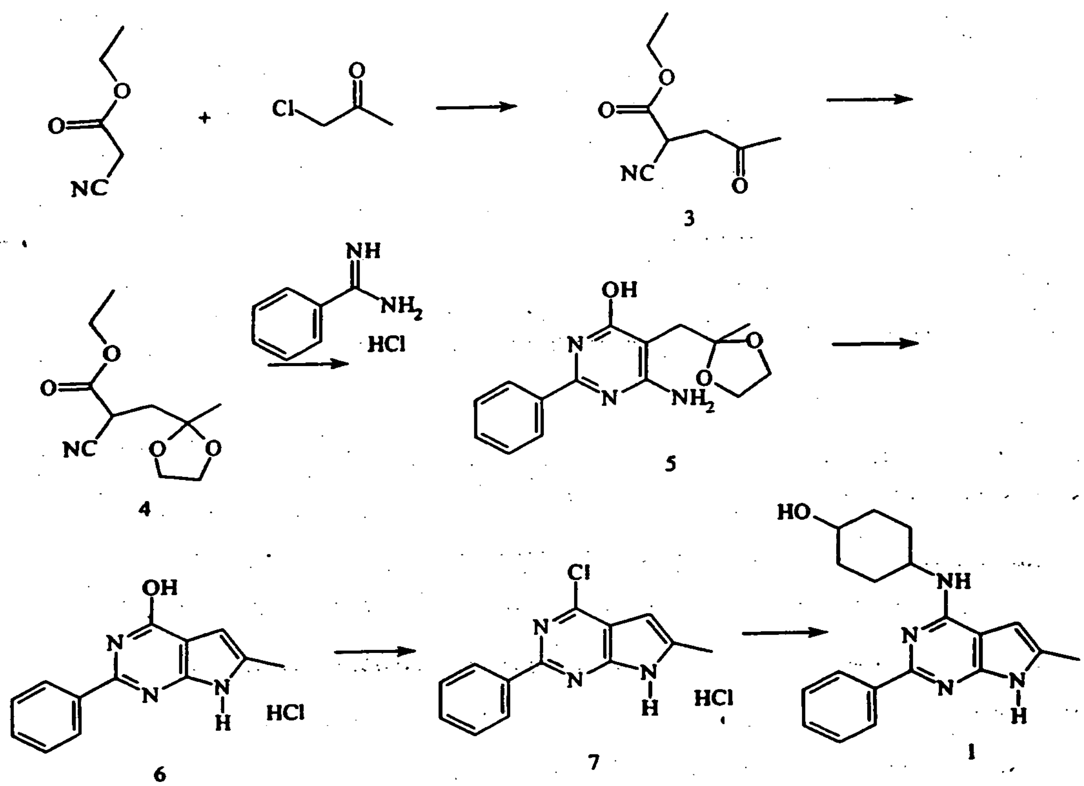
2

wherein R_5 and R_6 are as described above, *e.g.*, CH_3.

<u>**Specific Preparation of 6-methyl pyrrolopyrimidines:**</u>

The key reaction toward 6-methylpyrrolopyrimidines (1) [$R_5 = CH_3$] was cyclization of a cyanoacetate with benzamidine to a pyrimidine. It was believed methyl cyanoacetate would cyclize more efficiently with benzamidine to a pyrimidine than the corresponding ethyl ester. Therefore, transesterification and alkylation of ethyl cyanoacetate in the presence of NaOMe and an excess of an α-haloacetyl moiety, *e.g.*, chloroacetone, gave the desired methyl ester (3) in 79% yield (Scheme IV). The ketoester (3) was protected as the acetal (4) in 81% yield. A new cyclization method to the pyrimidine (5) was achieved with an amidine hydrochloride, *e.g.*, benzamidine hydrochloride, with 2 equivalents of DBU to afford the 5 in 54% isolated yield. This method improves the yield from 20% using the published conditions, which utilizes NaOMe during the cyclization with guanidine. Cyclization to the pyrrole-pyrimidine (6) was achieved via deprotection of the acetal in aqueous HCl in 78% yield. Reaction of (6) with phosphorous oxychloride at reflux gave the corresponding 4-chloro derivative (7). Coupling with *trans*-4-aminocyclohexanol in dimethyl sulfoxide at 135°C gave (1) in 57% from (7). One skilled in the art will appreciate that choice of reagents allows for great flexibility in choosing the desired substituent R_5.

Scheme IV

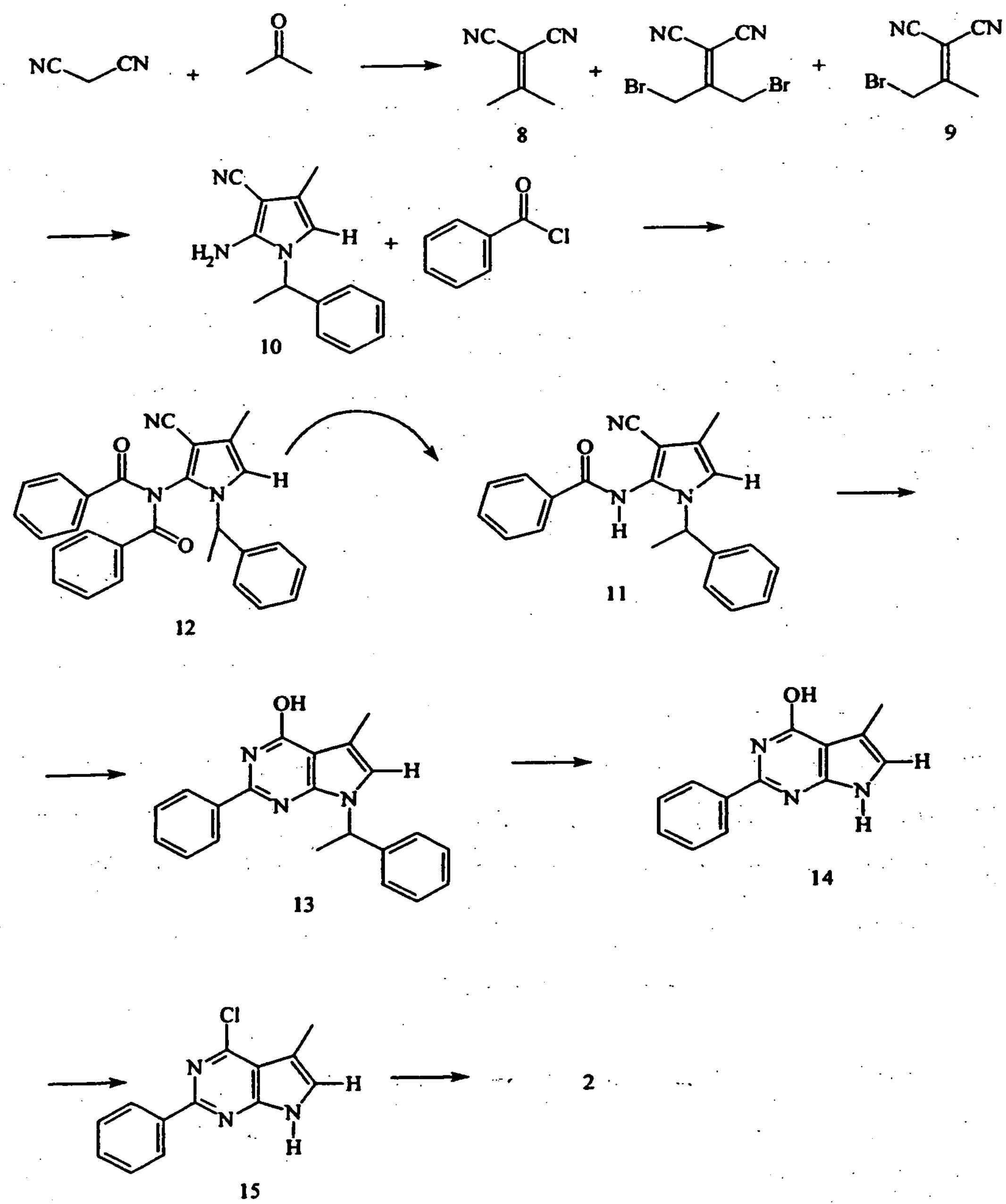


Specific Preparation of 5-methylpyrrolopyrimidines

Knoevengel condensation of malononitrile and an excess ketone, *e.g.*, acetone in refluxing benzene gave 8 in 50% yield after distillation. Bromination of 8 with *N*-bromosuccinimde in the presence of benzoyl peroxide in chloroform yielded a mixture of starting material, mono- (9), and di-brominated products (5/90/5) after distillation (70%). The mixture was reacted with an α-methylalkylamine or α-methylarylamine, *e.g.*, α-methylbenzylamine, to deliver the aminopyrrole (10). After passing through a short silica gel column, the partially purified amine (31% yield) was acylated with an acid chloride, *e.g.*, benzoyl chloride to deliver mono- (11), and diacylated (12) pyrroles, which were separated by flash chromatography. Acid hydrolysis of the disubstituted pyrrole (12) generated a combined yield of 29% for the acylpyrrole (11). Cyclization in the presence of concentrated sulphuric acid and DMF yielded (13) (23%), which was deprotected with polyphosphoric acid to (14). Reaction of (14) with phosphorous oxychloride at reflux gave the corresponding 4-chloro derivative (15). Coupling with

trans-4-aminocyclohexanol in dimethyl sulfoxide at 135°C gave (2) [$R_6 = CH_3$] in 30% from (14) (See Scheme V). One skilled in the art will appreciate that choice of reagents allows for great flexibility in choosing the desired substituent R_6.

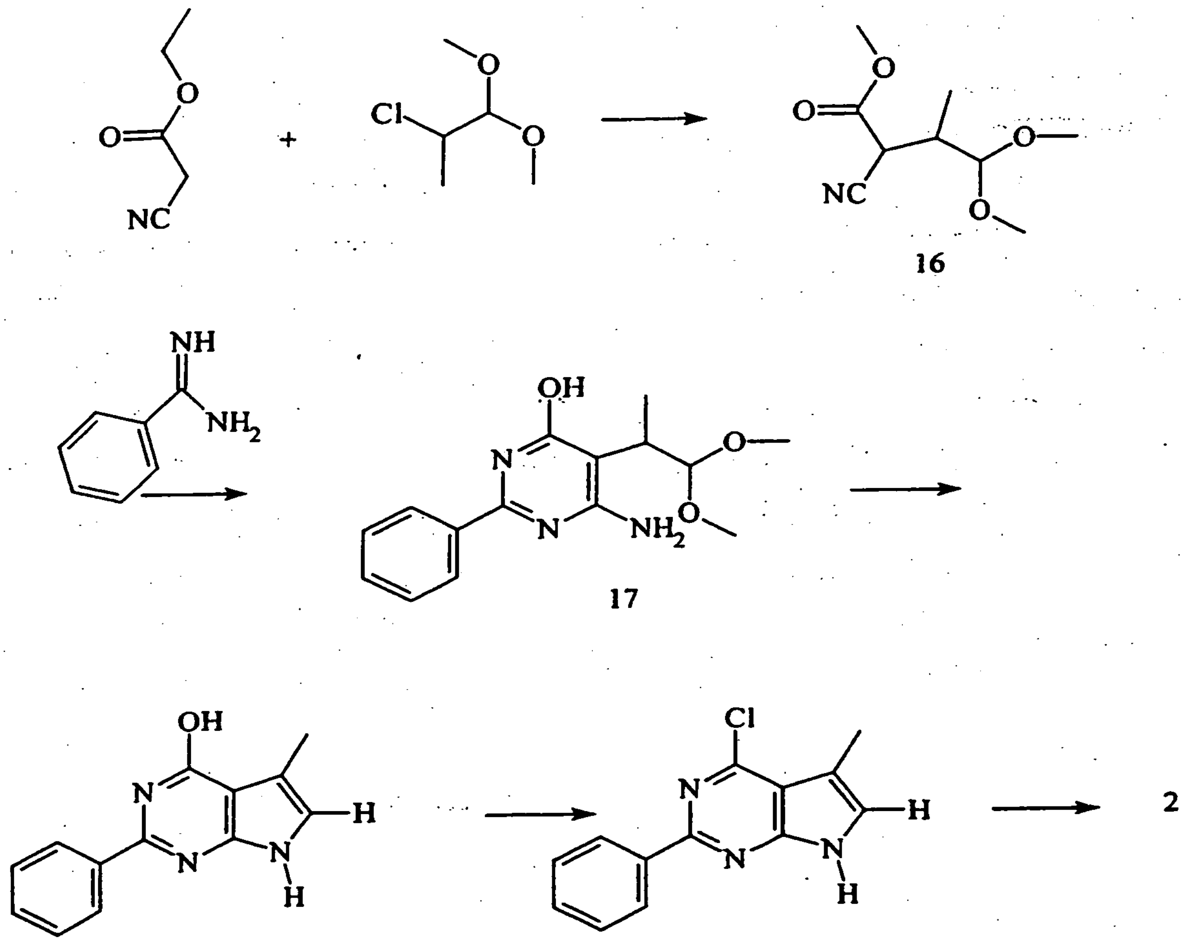
Scheme V



Alternative Synthetic Route to R_6-Substituted Pyrroles, *e.g.*, 5-methyl pyrrolopyrimidines:

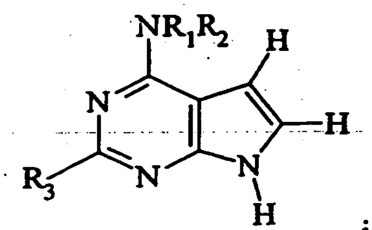
This alternative route to R_6-substituted pyrroles, *e.g.*, 5-methylpyrrolopyrimidines, involves transesterification and alkylation of ethyl cyanoacetate to (16) (Scheme VI). The condensation of (16) with benzamidine hydrochloride with 2 equivalents of DBU affords the pyrimidine (17). Cyclization to the pyrrole-pyrimidine (14) will be achieved via deprotection of the acetal in aqueous HCl. Reaction of (14) with phosphorous oxychloride at reflux gave the corresponding 4-chloro derivative (15). Coupling with *trans*-4-aminocyclohexanol in dimethyl sulfoxide at 135°C gives 2. This procedure reduces the number of synthetic reactions to the target compound (2) from 9 to 4 steps. Moreover, the yield is dramatically improved. Again, one skilled in the art will appreciate that choice of reagents allows for great flexibility in choosing the desired substituent R_6.

Scheme VI



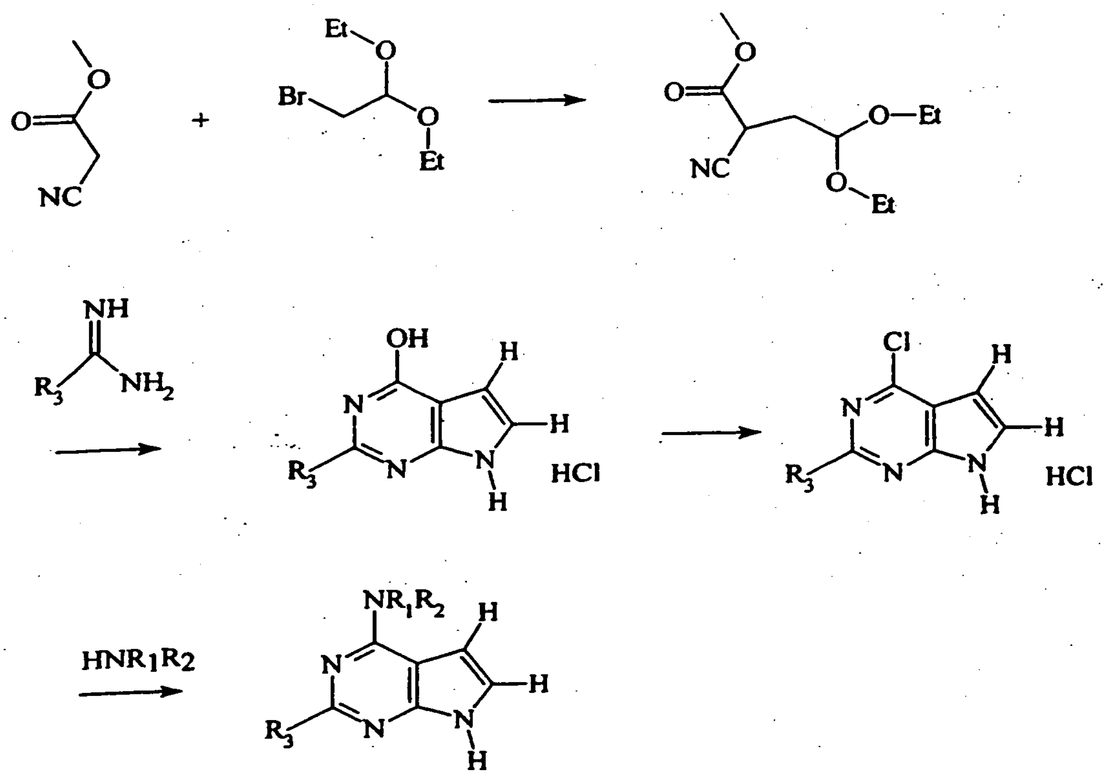
16 17 14 15 2

A general approach to prepare *des*-methyl pyrrole



is depicted in the following scheme (Scheme VII)

Scheme VII

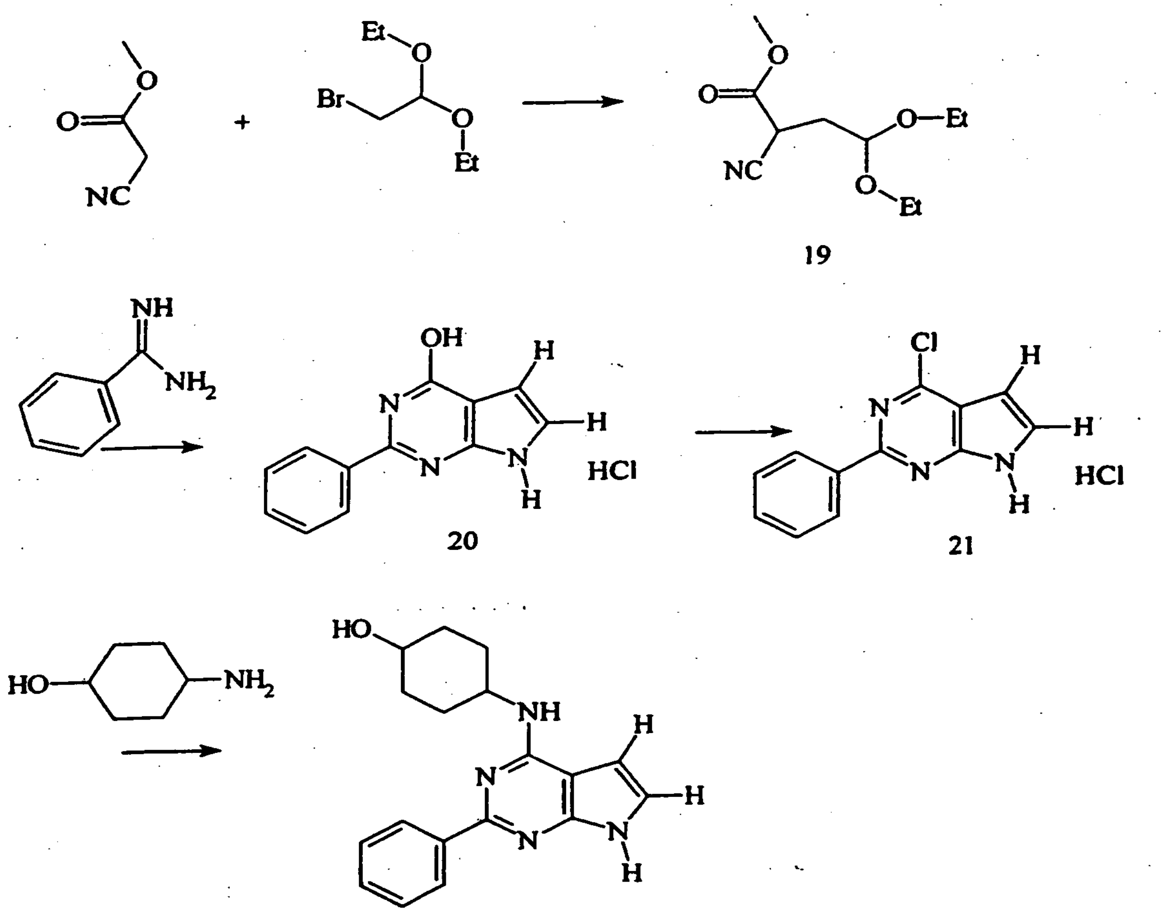


wherein R_1 through R_3 are defined as above.

Alkylation of an alkyl cyanoacetate with a diethyl acetal in the presence of a base afforded a cyano diethyl acetal which was treated with an amidine salt to produce a methyl pyrrolopyrimidine precursor. The precursor was chlorinated and treated with an amine to form the *des*-methyl pyrrolopyrimidine target as shown above.

For example, Scheme VIII depicts the synthesis of compound (18).

Scheme VIII



19

20

21

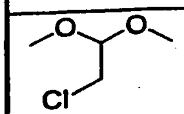
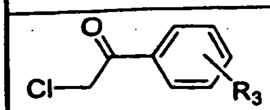
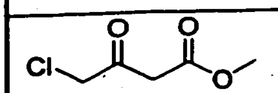
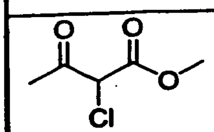
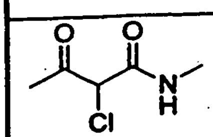
18

Commercially available methyl cyanoacetate was alkylated with bromoacetaldehyde diethyl acetal in the presence of potassium carbonate and NaI to yield (19). Cyclization to the pyrimidine (20) was achieved in two steps. Initially, the pyrimidine-acetal was formed via reaction of (19) with benzamidine hydrochloride with 2 equivalents of DBU. The resultant pyrimidine-acetal was deprotected without purification with aqueous 1 N HCl and the resultant aldehyde cyclized to the pyrrolo-pyrimidine (20), which was isolated by filtration. Reaction of (20) with phosphorous oxychloride at reflux afforded the corresponding 4-chloro derivative (21). Coupling of

the chloro derivative with *trans*-4-aminocyclohexanol in DMSO at 135° C gave compound (18) from compound (21).

Schemes II-VIII demonstrate that it is possible to functionalize the 5- and 6-position of the pyrrolopyrimidine ring. Through the use of different starting reagents and slight modifications of the above reaction schemes, various functional groups can be introduced at the 5- and 6-positions in formula (I) and (II). Table 2 illustrates some examples.

Table 2. Selected list of 5- and 6-substituted pyrrolopyrimidines.

Starting Reagent	R_5	R_6
	H	H
	H	Substituted Ar
	H	$CH_2C(O)OCH_3$
	$C(O)OCH_3$	CH_3
	$C(O)NHCH_3$	CH_3

The invention is further illustrated by the following examples which in no way should be construed as being further limiting. The contents of all references, pending patent applications and published patent applications, cited throughout this application, including those referenced in the background section, are hereby incorporated by reference. It should be understood that the models used throughout the examples are accepted models and that the demonstration of efficacy in these models is predictive of efficacy in humans.

Exemplification

Preparation 1:

A modification of the alkylation method of Seela and Lüpke was used.[1] To an ice-cooled (0°C) solution of ethyl cyanoacetate (6.58 g, 58.1 mmol) in MeOH (20 mL) was slowly added a solution of NaOMe (25% w/v; 58.1 mmol). After 10 min, chloroacetone (5 mL; 62.8 mmol) was slowly added. After 4 h, the solvent was removed. The brown oil was diluted the EtOAc (100 mL) and washed with H_2O (100 mL). The organic fraction was dried, filtered, and concentrated to a brown oil (7.79 g; 79%). The oil (3) (Scheme IV) was a mixture of methyl/ethyl ester products (9/1), and was used without further purification. ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.24 (q, J = 7.2 Hz, OCH_2), 3.91 (dd, 1H, J = 7.2, 7.0 Hz, CH), 3.62 (s, 3H, OCH_3), 3.42 (dd, 1H, J = 15.0, 7.1 Hz, 1 x CH_2); 3.02 (dd, 1H, J = 15.0, 7.0 Hz, 1 x CH_2); 2.44 (s, 3H, CH_3), 1.26 (t, J = 7.1 Hz, ester-CH_3).

[1] Seela, F.; Lüpke, U. Chem. Ber. 1977, 110, 1462-1469.

Preparation 2:

The procedure of Seela and Lüpke was used.[1] Thus, protection of the ketone (3) (Scheme IV; 5.0 g, 32.2 mmol) with ethylene glycol (4 mL, 64.4 mmol) in the presence of TsOH (100 mg) afforded (4) as an oil (Scheme IV; 5.2 g, 81.0) after flash chromatography (SiO_2; 3/7 EtOAc/Hex, R_f 0.35). Still contains ~5% ethyl ester: ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.24 (q, J = 7.2 Hz, OCH_2), 3.98 (s, 4H, 2 x acetal-CH_2), 3.79 (s, 3H, OCH_3), 3.62 (dd, 1H, J = 7.2, 7.0 Hz, CH), 2.48 (dd, 1H, J = 15.0, 7.1 Hz, 1 x CH_2), 2.32 (dd, 1H, J = 15.0, 7.0 Hz, 1 x CH_2); 1.35 (s, 3H, CH_3), 1.26 (t, J = 7.1 Hz, ester-CH_3); MS (ES): 200.1 (M^++1).

[1] Seela, F.; Lüpke, U. Chem. Ber. 1977, 110, 1462-1469.

Preparation 3:

A solution of acetal (4) (Scheme IV, 1 g, 5.02 mmol), benzamidine (786 mg, 5.02 mmol), and DBU (1.5 mL, 10.04 mmol) in dry DMF (15 mL) was heated to 85°C for 15 h. The mixture was diluted with $CHCl_3$ (30 mL) and washed with 0.5 N NaOH

(10 mL) and H_2O (20 mL). The organic fraction was dried, filtered and concentrated to a brown oil. Flash chromatography (SiO_2; 1/9 EtOAc/CH_2Cl_2, *Rf* 0.35) was attempted, but material crystallized on the column. The silica gel was washed with MeOH. Fractions containing the product (5) (Scheme IV) were concentrated and used without further purification (783 mg, 54.3%): ^1H NMR (200 MHz, $CDCl_3$) δ 8.24 (m, 2H, Ar-H), 7.45 (m, 3H, Ar-H), 5.24 (br s, 2H, NH_2), 3.98 (s, 4H, 2 x acetal-CH_2), 3.60-3.15 (m, 2H, CH_2), 1.38 (s, 3H, CH_3); MS (ES): 288.1 (M^++1).

Preparation of compound (20) (Scheme VIII): A solution of acetal (19) (4.43 g, 20.6 mmol)[1], benzamine hydrochloride (3.22 g, 20.6 mmol), and DBU (6.15 mL, 41.2 mmol) in dry DMF (20 mL) was heated to 85°C for fifteen hours. The mixture was diluted with 100mL of $CHCl_3$, and washed with H_2O (2 x 50 mL). The organic fraction was dried, filtered, and concentrated to a dark brown oil. The dark brown oil was stirred in 1N HCl (100 mL) for 2 hours at room temperature. The resulting slurry was filtered yielding the HCl salt of (20) as a tan solid (3.60 g, 70.6%); ^1H NMR (200 MHz, DMSO-d6) 11.92 (s 1H), 8.05 (m, 2H, Ar-H), 7.45 (m, 3H, Ar-H), 7.05 (s, 1H, pyrrole-H); MS(ES): 212.1 (M^++1).

Preparation 4:

A solution of acetal (5) (700 mg, 2.44 mmol) in 1 N HCl (40 mL) was stirred for 2 h at RT. The resultant slurry was filtered yielding the HCl salt of 2-phenyl-6-methyl-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one as a tan solid (498 mg, 78.0%): ^1H NMR (200 MHz, DMSO-d_6) δ 11.78 (s, 1H), 8.05 (m, 2H, Ar-H), 7.45 (m, 3H, Ar-H), 6.17 (s, 1H, pyrrole-H), 2.25 (s, 3H, CH_3); MS (ES): 226.1 (M^++1).

Preparation 5:

A modification of the Chen *et al.* cyclization method was used.[1] To an ice-cooled (0°C) solution of bromide (9), (Scheme V; 20.0 g, 108 mmol; 90% pure) in isopropyl alcohol (60 mL) was slowly added a solution of α-methylbenzylamine (12.5 mL, 97.3 mmol). The black solution was allowed to warm to RT and stir for 15 h. The mixture was diluted with EtOAc (200 mL) and washed with 0.5 N NaOH (50 mL). The

organic fraction was dried, filtered, and concentrated to a black tar (19.2 g; 94%). The residue was partially purified by flash chromatography (SiO_2; 4/96 $MeOH/CH_2Cl_2$, *Rf* 0.35) to a black solid (6.38 g, 31%) as the compound *dl*-1-(1-phenylethyl)-2-amino-3-cyano-4-methylpyrrole: MS (ES): 226.1 (M^++1).

[1] Chen, Y. L.; Mansbach, R. S.; Winter, S. M.; Brooks, E.; Collins, J.; Corman, M. L.; Dunaiskis, A. R.; Faraci, W. S.; Gallaschun, R. J.; Schmidt, A.; Schulz, D. W. *J. Med. Chem.* 1997, 40, 1749-1754.

Preparation 6:

To a solution of *dl*-1-(1-phenylethyl)-2-amino-3-cyano-4,5-dimethylpyrrole[1] (14.9 g, 62.5 mmol) and pyridine (10.0 mL) in dichloromethane (50.0 mL) was added benzoyl chloride (9.37 g, 66.7 mmol) at 0°C. After stirring at 0°C for 1 hr, hexane (10.0 mL) was added to help precipitation of product. Solvent was removed *in vacuo* and the solid was recrystallized from $EtOH/H_2O$ to give 13.9 g (65%) of *dl*-1-(1-phenylethyl)-2-phenylcarbonylamino-3-cyano-4,5-dimethylpyrrole. mp 218-221°C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.72 (s, 3H), 1.76 (d, J = 7.3 Hz, 3H), 1.98 (s, 3H), 5.52 (q, J = 7.3 Hz, 1H), 7.14-7.54 (m, 9H), 7.68-7.72 (dd, J = 1.4 Hz, 6.9 Hz , 2H), 10.73 (s, 1H); MS (ES): 344.4 (M^++1).

[1] Liebigs Ann. Chem. 1986, 1485-1505.

The following compounds were obtained in a similar manner as that of

Preparation 6:

dl-1-(1-phenylethyl)-2-(3-pyridyl)carbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.83 (d, J = 6.8 Hz, 3H), 2.02 (s, 3H), 2.12 (s, 3H), 5.50 (q, J = 6.8 Hz, 1H), 7.14-7.42 (m, 5H), 8.08 (m, 2H), 8.75 (m, 3H); MS (ES): 345.2 (M^++1).

dl-1-(1-phenylethyl)-2-(2-furyl)carbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.84 (d, J = 7.4 Hz, 3H), 1.92 (s, 3H), 2.09 (s, 3H), 5.49 (q, J = 7.4 Hz, 1H), 6.54 (dd, J = 1.8 Hz, 3.6 Hz, 1H), 7.12-7.47 (m, 7H); MS (ES): 334.2 (M^++1), 230.1.

dl-1-(1-phenylethyl)-2-(3-furyl)carbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.80 (d, J = 7 Hz 3H), 1.89 (s, 3H), 2.05 (s, 3H), 5.48 (q, J = 7 Hz, 1H), 6.59 (s, 1H), 7.12-7.40 (m, 6H), 7.93 (s, 1H); MS (ES): 334.1 (M^++1), 230.0.

dl-1-(1-phenylethyl)-2-cyclopentylcarbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.82 (d, J = 7.4 Hz, 3H), 1,88 (s, 3H), 2.05 (s, 3H), 1.63-1.85 (m, 8H), 2.63 (m, 1H), 5.43 (q, J = 7.4 Hz, 1H), 6.52 (s, 1H), 7.05-7.20 (m, 5H); MS (ES): 336.3 (M^++1).

dl-1-(1-phenylethyl)-2-(2-thieyl)carbonylamino-3-cyano-4,5-dimethylpyrrole, ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.82 (d, J = 6.8 Hz, 3H), 1.96 (s, 3H), 2.09 (s, 3H), 5.49 (q, J= 6.8 Hz, 1H), 7.05-7.55 (m, 8H); MS (ES): 350.1 (M^++1), 246.0.

dl-1-(1-phenylethyl)-2-(3-thienyl)carbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.83 (d, J = 7.0 Hz, 3H), 1.99 (s, 3H), 2.12 (s, 3H), 5.49 (q, J = 7.0 Hz, 1H), 6.90 (m, 1H), 7.18–7.36 (m, 6H), 7.79 (m, 1H); MS (ES): 350.2 (M^++1), 246.1.

dl-1-(1-phenylethyl)-2-(4-fluorophenyl)carbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.83 (d, J = 7.4 Hz, 3H), 1.96 (s, 3H), 2.08 (s, 3H), 5.51 (q, J = 7.4 Hz, 1H), 7.16-7.55 (m, 9H); MS (ES): 362.2 (M^++1), 258.1.

dl-1-(1-phenylethyl)-2-(3-fluorophenyl)carbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.83 (d, J = 7.4 Hz 3H), 1.97 (s, 3H), 2.10(s, 3H), 5.50 (q, J = 7.4 Hz, 1H), 7.05-7.38 (m, 7 H), 7.67-7.74 (m, 2H); MS (ES): 362.2 (M^++1), 258.1.

dl-1-(1-phenylethyl)-2-(2-fluorophenyl)carbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.85 (d, J = 7.2 Hz, 3H), 1.94 (s, 3H), 2.11 (s, 3H), 5.50 (q, J = 7.2 hz, 1H), 7.12-7.35 (m, 6H), 7.53 (m, 1H), 7.77 (m, 1H), 8.13 (m, 1H); MS (ES): 362.2(M^++1), 258.0.

dl-1-(1-phenylethyl)-2-isoproylcarbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.19 (d, J = 7.0 Hz, 6H), 1.82(d, J = 7.2 Hz, 3H), 1.88 (s, 3H), 2.06 (s, 3H), 2.46 (m, 1H), 5.39 (m, J = 7.2 Hz, 1H), 6.64 (s, 1H), 7.11-7.36 (m, 5H); MS (ES): 310.2 (M^++1), 206.1 .

In the case of acylation of *dl*-1-(1-phenylethyl)-2-amino-3-cyano-4-methylpyrrole, monoacylated *dl*-1-(1-phenylethyl)-2-benzoylamino-3-cyano-4-dimethylpyrrole and diacylated pyrrole *dl*-1-(1-phenylethyl)-2-dibenzoylamino-3-cyano-4-methylpyrrole were obtained. Monoacylated pyrrole: ^{1}H NMR (200 MHz, CDCl3) δ 7.69 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.58-7.12 (m, 8H, Ar-H), 6.18 (s, 1H, pyrrole-H), 5.52 (q, 1H, *J* = 7.2 Hz, C<u>H</u>-CH3), 2.05 (s, 3H, pyrrole-CH3), 1.85 (d, 3H, *J* = 7.2 Hz, CH-C<u>H</u>3); MS (ES): 330.2 (M^++1); Diacylated pyrrole: ^{1}H NMR (200 MHz, CDCl3) δ 7.85 (d, 2H, *J* = 7.7 Hz, Ar-H), 7.74 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.52-7.20 (m, 9H, Ar-H), 7.04 (m, 2H, Ar-H), 6.21 (s, 1H, pyrrole-H), 5.52 (q, 1H, *J* = 7.2 Hz, C<u>H</u>-CH3), 1.77 (d, 3H, *J* = 7.2 Hz, CH-C<u>H</u>3), 1.74 (s, 3H, pyrrole-CH3); MS (ES): 434.1 (M^++1).

<u>Preparation 7:</u>

To a solution of *dl*-1-(1-phenylethyl)-2-phenylcarboxyamido-3-cyano-4,5-dimethylpyrrole (1.0 g, 2.92 mmol) in methanol (10.0 mL) was added concentrated sulfuric acid (1.0 mL) at 0°C. The resulted mixture was refluxed for 15 hr and cooled down to room temperature. The precipitate was filtered to give 0.48 g (48%) of *dl*-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.02 (d, J = 7.4 Hz, 3H), 2.04 (s, 3H), 2.41 (s, 3H), 6.25 (q, J = 7.4 Hz, 1H), 7.22-7.50 (m, 9H), 8.07-8.12 (dd, J = 3.4 Hz, 6.8 Hz, 2H), 10.51 (s, 1H); MS (ES): 344.2 (M^++1).

The following compounds were obtained in a similar manner as that of <u>Preparation 7:</u>

dl-5,6-dimethyl-2-(3-pyridyl)-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.03 (d, J = 7.2 Hz, 3H), 2.08 (s, 3H), 2.42 (s, 3H), 6.24 (q, J = 7.2 Hz, 1H), 7.09-7.42 (m, 5H), 8.48 (m, 2H), 8.70 (m, 3H); MS (ES): 345.1 (M^++1).

dl-5,6-dimethyl-2-(2-furyl)-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.98 (d, J = 7.8 Hz, 3H), 1.99 (s, 3H), 2.37 (s, 3H), 6.12 (q, J = 7.8 Hz, 1H), 6.48 (dd, J=1.8 Hz, 3.6 Hz, 1H), 7.17-7.55 (m, 7H), 9.6 (s, 1H); MS (ES): 334.2 (M^++1).

dl-5,6-dimethyl-2-(3-furyl)-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.99 (d, J = 7 Hz, 3H), 2.02 (s, 3H), 2.42 (s, 3H), 6.24 (q, J = 7 Hz, 1H), 7.09 (s, 1H), 7.18-7.32 (m, 5H), 7.48 (s, 1H), 8.51 (s, 1H); MS (ES): 334.2 (M^++1).

dl-5,6-dimethyl-2-cyclopentyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.95 (d, J = 7.4 Hz, 3H), 2.00 (s, 3H), 2.33 (s, 3H), 1.68-1.88 (m, 8H), 2.97 (m, 1H), 6.10 (q, J = 7.4 Hz, 1H), 7.16-7.30 (m, 5H), 9.29 (s, 1H); MS (ES): 336.3 (M^++1).

dl-5,6-dimethyl-2-(2-thienyl)-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.02(d, J = 7.2 Hz, 3H), 2.06 (s, 3H), 2.41 (s, 3H), 6.13 (q, J = 7.2 Hz, 1H), 7.12 (dd, J = 4.8, 2.8 Hz, 1H), 7.26-7.32 (m, 5H), 7.44 (d, J = 4.8 Hz, 1H), 8.01 (d, J = 2.8 Hz, 1H) 11.25 (s, 1H); MS (ES): 350.2 (M^++1).

dl-5,6-dimethyl-2-(3-thienyl)-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.00 (d, J = 7.4 Hz, 3H), 2.05 (s, 3H), 2.43 (s, 3H), 6.24(q, J = 7.4 Hz, 1H), 7.24-7.33 (m, 5H), 7.33-7.39 (m, 1H), 7.85 (m, 1H), 8.47 (m, 1H), 12.01 (s, 1H); MS (ES): 350.2 (M^++1).

dl-5,6-dimethyl-2-(4-fluorophenyl)-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.01 (d, J = 6.8 Hz, 3H), 2.05 (s, 3H), 2.42 (s, 3H), 6.26 (q, J = 6.8 Hz, 1H), 7.12-7.36 (m, 7H), 8.23-8.30 (m, 2H), 11.82 (s, 1H); MS (ES): 362.3 (M^++1).

dl-5,6-dimethyl-2-(3-fluorophenyl)-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.02 (d, J = 7.4 Hz, 3H), 2.06 (s, 3H), 2.44 (s, 3H), 6.29 (q, J = 7.4 Hz, 1H), 7.13-7.51(m, 7H), 8.00-8.04 (m, 2H), 11.72 (s, 1H); MS (ES): 362.2 (M^++1).

dl-5,6-dimethyl-2-(2-fluorophenyl)-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.00(d, J = 7.2 Hz, 3H), 2.05 (s, 3H), 2.38 (s, 3H), 6.24 (q, J = 7.2 Hz, 1H), 7.18 – 7.45 (m, 8 H), 8.21 (m, 1H), 9.54 (s, 1H); MS (ES): 362.2 (M^++1).

dl-5,6-dimethyl-2-isopropyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30 (d, J = 6.8 Hz, 3H), 1.32 (d, J = 7.0 Hz, 3H), 2.01 (s, 3H), 2.34 (s, 3H), 2.90 (m, 1H), 6.13 (m, 1H), 7.17-7.34 (m, 5H), 10.16 (s, 1H); MS (ES): 310.2 (M^++1).

Preparation 8:

A solution of *dl*-1-(1-phenylethyl)-2-benzoylamino-3-cyano-4-dimethylpyrrole (785 mg, 2.38 mmol) with concentrated H_2SO_4 (1 mL) in DMF (13 mL) was stirred at 130°C for 48 h. The black solution was diluted with $CHCl_3$ (100 mL) and washed with 1 N NaOH (30 mL), and brine (30 mL). The organic fraction was dried, filtered, concentrated, and purified by flash chromatography (SiO_2; 8/2 EtOAc/Hex, *Rf* 0.35) to a brown solid (184 mg, 24%) as *dl*-5-methyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.18 (m, 2H, Ar-H), 7.62-7.44 (m, 3H, Ar-H), 7.40-7.18 (m, 5H, Ar-H), 6.48 (s, 1H, pyrrole-H),

6.28 (q, 1H, J = 7.2 Hz, C<u>H</u>-CH_3), 2.18 (s, 3H, pyrrole-CH_3), 2.07 (d, 3H, J = 7.2 Hz, CH-C<u>H</u>3); MS (ES): 330.2 (M^+ + 1).

<u>**Preparation 9:**</u>

A mixture of *dl*-1-(1-phenylethyl)-2-amino-3-cyano-4,5-dimethylpyrrole (9.60 g, 40.0 mmol) and of formic acid (50.0 mL, 98%) was refluxed for 5 hr. After cooling down to room temperature and scratching the sides of flask, copious precipitate was formed and filtered. The material was washed with water until washings showed neutral pH to give *dl*-5,6-dimethyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^1H NMR (200 MHz, $CDCl_3$) δ 1.96 (d, J = 7.4 hz, 3H), 2.00 (s, 3H), 2.38 (s, 3H), 6.21 (q, J = 7.4 Hz, 1H), 7.11-7.35 (m, 5H), 7.81 (s, 1H), 11.71 (s, 1H); MS (ES): 268.2 (M^++1).

<u>**Preparation 10:**</u>

dl-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one (1.0 g, 2.91 mmol) was suspended in polyphosphoric acid (30.0 mL). The mixture was heated at 100°C for 4 hr. The hot suspension was poured onto ice water, stirred vigorously to disperse suspension, and basified to pH 6 with solid KOH. The resulting solid was filtered and collected to give 0.49 g (69%) of 5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^1H NMR (200 MHz, DMSO-d_6) δ 2.17 (s, 3H), 2.22 (s, 3H), 7.45 (br, 3H), 8.07 (br, 2H,), 11.49 (s, 1H), 11.82 (s, 1H); MS (ES): 344.2 (M^++1).

The following compounds were obtained in a similar manner as that of <u>Preparation 10:</u>

5-methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. MS (ES): 226.0 (M^++1).

5,6-dimethyl-2-(3-pyridyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. MS (ES): 241.1 (M^++1).

5,6-dimethyl-2-(2-furyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.13 (s, 3H), 2.18 (s, 3H), 6.39 (dd, J = 1.8, 3.6 Hz, 1H), 6.65 (dd, J = 1.8 Hz, 3.6 Hz, 1H), 7.85 (dd, J = 1.8, 3.6 Hz, 1H,), 11.45 (s, 1H), 11.60 (s, 1H); MS (ES): 230.1 (M^++1).

5,6-dimethyl-2-(3-furyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.14 (s, 3H), 2.19 (s, 3H), 6.66 (s, 1H), 7.78 (s, 1H), 8.35 (s, 1H), 11.3 (s, 1H), 11.4 (s, 1H); MS (ES): 230.1 (M^++1).

5,6-dimethyl-2-cyclopentyl-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.57-1.91 (m, 8 H), 2.12 (s, 3H), 2.16 (s, 3H), 2.99 (m, 1H), 11.24 (s, 1H), 11.38 (s, 1H); MS (ES): 232.2 (M^++1).

5,6-dimethyl-2-(2-thienyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.14 (s, 3H), 2.19 (s, 3H), 7.14 (dd, J = 3.0, 5.2 Hz, 1H), 7.70 (d, J = 5.2 Hz 1H), 8.10 (d, J=3.0 Hz, 1H), 11.50 (s, 1H); MS (ES): 246.1 (M^++1).

5,6-dimethyl-2-(3-thienyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.17 (s, 3H), 2.21(s, 3H), 7.66(m, 1H), 7.75 (m, 1H), 8.43 (m, 1H), 11.47 (s, 1H), 11.69 (s, 1H); MS (ES): 246.1 (M^++1).

5,6-dimethyl-2-(4-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.17 (s, 3H), 2.21 (s, 3H), 7.31 (m, 2H), 8.12 (m, 2H), 11.47 (s, 1H); MS (ES): 258.2 (M^++1).

5,6-dimethyl-2-(3-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.18 (s, 3H), 2.21 (s, 3H), 7.33 (m, 1H), 7.52 (m, 1H), 7.85-7.95 (m, 2H), 11.56 (s, 1H), 11.80 (s, 1H); MS (ES): 258.1 (M^++1).

5,6-dimethyl-2-(2-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.18 (s, 3H), 2.22 (s, 3H), 7.27-7.37 (m, 2H), 7.53 (m 1H), 7.68 (m, 1H), 11.54 (s, 1H), 11.78 (s, 1H); MS (ES): 258.1 (M^++1).

5,6-dimethyl-2-isopropyl-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.17 (d, J= 6.6 Hz, 6H), 2.11 (s, 3H), 2.15 (s, 3H), 2.81 (m, 1H), 11.20 (s, 1H), 11.39 (s, 1H); MS (ES): 206.1 (M^++1).

5,6-dimethyl-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.13 (s, 3H), 2.17 (s, 3H), 7.65 (s, 1H); MS (ES): 164.0 (M^++1).

Preparation 11:

A solution of 5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one (1.0 g, 4.2 mmol) in phosphorus oxychloride (25.0 mL) was refluxed for 6 hr and then concentrated *in vacuo* to dryness. Water was added to the residue to induce crystallization and the resulting solid was filtered and collected to give 0.90 g (83%) of 4-chloro-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.33 (s, 3H), 2.33 (s, 3H), 7.46-7.49 (m, 3H), 8.30-8.35 (m, 2H), 12.20 (s, 1H); MS (ES): 258.1 (M^++1).

The following compounds were obtained in a similar manner as that of Preparation 11:

4-chloro-5-methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 244.0 (M^++1).

4-chloro-6-methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 244.0 (M^++1).

4-chloro-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d6) 8.35 (2, 2H), 7.63 (br s, 1H), 7.45 (m, 3H), 6.47 (br s, 1H); MS (ES): 230.0 (M^++1).

4-chloro-5,6-dimethyl-2-(3-pyridyl)-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 259.0 (M^++1).

4-chloro-5,6-dimethyl-2-(2-furyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.35 (s, 3H), 2.35 (s, 3H), 6.68 (dd, J = 1.8, 3.6 Hz, 1H), 7.34 (dd, , J = 1.8 Hz, 3.6 Hz, 1H), 7.89 (dd, J = 1.8, 3.6 Hz, 1H); MS (ES): 248.0 (M^++1).

4-chloro-5,6-dimethyl-2-(3-furyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.31 (s, 3H), 2.31 (s, 3H), 6.62 (s, 1H), 7.78 (s, 1H), 8.18 (s, 1H), 12.02 (s, 1H); MS (ES): 248.1 (M^++1

4-chloro-5,6-dimethyl-2-cyclopentyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.61- 1.96 (m, 8H), 2.27 (s, 3H), 2.27 (s, 3H), 3.22 (m, 1H), 11.97 (s, 1H); MS (ES): 250.1 (M^++1).

4-chloro-5,6-dimethyl-2-(2-thienyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.29 (s, 3H), 2.31 (s, 3H), 7.14 (dd, J = 3.1 Hz, 4.0 Hz, 1H), 7.33 (d, J = 4.9 Hz, 1H), 7.82 (d, J = 3.1 Hz, 1H), 12.19 (s, 1H); MS (ES): 264.1 (M^++1).

4-chloro-5,6-dimethyl-2-(3-thienyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.32 (s, 3H), 2.32 (s, 3H), 7.62 (dd, J = 3.0, 5.2 Hz, 1H), 7.75 (d, J = 5.2 Hz, 1H), 8.20 (d, J = 3.0 Hz, 1H); MS (ES): 264.0 (M^++1).

4-chloro-5,6-dimethyl-2-(4-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.33(s, 3H), 2.33 (s, 3H), 7.30 (m, 2H), 8.34 (m, 2H), 12.11 (s, 1H); MS (ES): 276.1. (M^++1).

4-chloro-5,6-dimethyl-2-(3-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.31(s, 3H), 2.33 (s, 3H), 7.29(m, 1H), 7.52 (m, 1H), 7.96 (m, 1H), 8.14(m, 1H), 11.57 (s, 1H); MS (ES): 276.1 (M^++1).

4-chloro-5,6-dimethyl-2-(2-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.34 (s, 3H), 2.34 (s, 3H), 7.33 (m, 2H), 7.44 (m, 1H), 7.99 (m, 1H), 12.23 (s, 1H); MS (ES): 276.1 (M^++1).

4-chloro-5,6-dimethyl-2-isopropyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.24 (d, J = 6.6 Hz, 6H), 2.28 (s, 3H), 2.28 (s, 3H), 3.08 (q, J = 6.6 Hz, 1H), 11.95 (s, 1H); MS (ES): 224.0 (M^++1).

4-chloro-5,6-dimethyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.31 (s, 3H), 2.32 (s, 3H), 8.40 (s, 1H); MS (ES): 182.0 (M^++1).

dl-4-chloro-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidine.

Preparation 12:

To a solution of *dl*-1,2-diaminopropane (1.48 g, 20.0 mmol) and sodium carbonate (2.73 g, 22.0 mmol) in dioxane (100.0 mL) and water (100.0 mL) was added di-*tert*-dicarbonate (4.80 g, 22.0 mmol) at room temperature. The resulted mixture was stirred for 14 hr. Dioxane was removed *in vacuo*. The precipitate was filtered off and the filtrate was concentrated *in vacuo* to dryness. The residue was triturated with EtOAc and then filtered. The filtrate was concentrated *in vacuo* to dryness to give a mixture of *dl*-1-amino-2-(1,1-dimethylethoxy)carbonylamino-propane and *dl*-2-amino-1-(1,1-dimethylethoxy)carbonylamino-propane which were not separable by normal chromatography method. The mixture was used for the reaction in Example 8.

Preparation 13:

To solution of Fmoc-β-Ala-OH (1.0 g, 3.212 mmol) and oxalyl chloride (0.428 g, 0.29 mL, 3.373 mmol) in dichloromethane (20.0 mL) was added a few drops of N,N-dimethylformamide at 0°C. The mixture was stirred at room temperature for 1 hr followed by addition of cyclopropylmethylamine (0.229 g, 0.28 mL, 3.212 mmol) and triethylamine (0.65 g, 0.90 mL, 6.424 mmol). After 10 min, the mixture was treated with

1 M hydrochloride (10.0 mL) and the aqueous mixture was extracted with dichloromethane (3 x 30.0 mL). The organic solution was concentrated *in vacuo* to dryness. The residue was treated with a solution of 20% piperidine in N,N-dimethylforamide (20.0 mL) for 0.5 hr. After removal of the solvent *in vacuo,* the residue was treated with 1 M hydrochloride (20.0 mL) and ethyl acetate (20.0 mL). The mixture was separated and the aqueous layer was basified with solid sodium hydroxide to pH = 8. The precipitate was removed by filtration and the aqueous solution was subjected to ion exchange column eluted with 20% pyridine to give 0.262 g (57%) of N-cyclopropylmethyl β-alanine amide. ^{1}H NMR (200 MHz, CD_3OD) δ 0.22 (m, 2H), 0.49 (m, 2H), 0.96 (m, 2H), 2.40 (t, 2H), 2.92 (t, 2H), 3.05 (d, 2H); MS (ES): 143.1 (M^++1).

Preparation 14:

N-*tert*-butoxycarbonyl-*trans*-1,4-cyclohexyldiamine.

trans-1,4-cyclonexyldiamine (6.08 g, 53.2 mmol) was dissolved in dichloromethane (100mL). A solution of di-t-butyldicarbonate (2.32 g, 10.65 mmol in 40 mL dichloromethane) was added via cannula. After 20 hours, the reaction was partitioned between $CHCl_3$ and water. The layers were separated and the aqueous layer was extracted with $CHCl_3$ (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to yield 1.20 g of a white solid (53%). ^{1}H-NMR (200MHz, $CDCl_3$): δ 1.0-1.3 (m, 4H), 1.44 (s, 9H), 1.8 –2.1 (m, 4H), 2.62 (brm, 1H), 3.40 (brs, 1H), 4.37 (brs, 1H0; MS (ES): 215.2 (M^++1).

4-(N-acetyl)-N-*tert*-butoxycarbonyl-*trans*-1,4-cyclohexyldiamine.

N-*tert*-butoxycarbonyl-*trans*-1,4-cyclohexyldiamine (530 mg, 2.47 mmol) was dissolved in dichloromethane (20 mL). Acetic anhydride (250 mg, 2.60 mmol) was added dropwise. After 16 hours, the reaction was diluted with water and $CHCl_3$. The layers were separated and the aqueous layer was extracted with $CHCl_3$ (3x).). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Recrystallization ($EtOH/H_2O$) yielded 190 mg of white crystals (30%). ^{1}H NMR (200 MHz, $CDCl_3$): δ

0.9 – 1.30 (m, 4H), 1.43 (s, 9H), 1.96-2.10 (m, 7H), 3.40 (brs, 1H), 3.70 (brs, 1H), 4.40 (brs, 1H), 4.40 (brs, 1H); MS (ES): 257.2 (M^++1), 242.1 (M^+ - 15), 201.1 (M^+ - 56).

4-(4-*trans*-acetamidocyclohexyl)amino-5,6-dimethyl-2-phenyl-7H-(1-phenylethyl) pyrrolo[2,3d]pyrimidine.

4-(N-acetyl)-N-*tert*-butoxycarbonyl-*trans*-1,4-cyclohexyldiamine (190 mg, 0.74 mmol), was dissolved in dichloromethane (5 mL) and diluted with TFA (6 ml). After 16 hours, the reaction was concentrated. The crude solid, DMSO (2mL), $NaHCO_3$ (200 mg, 2.2 mmol) and 4-chloro-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine (35 mg, 0.14 mmol) were combined in a flask and heated to 130 °C. After 4.5 hours, the reaction was cooled to room temperature and diluted with EtOAc and water. The layers were separated and the aqueous layer was extracted with EtOAc (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Chromatography (silica preparatory plate; 20:1 $CHCl_3$:EtOH) yielded 0.3 mg of a tan solid (1% yield). MS (ES): 378.2 (M^++1).

4-(N-methanesulfonyl)-N-*tert*-butoxycarbonyl-*trans*-1,4-cyclohexyldiamine.

trans-1,4-cyclohexyldiamine (530 mg, 2.47 mmol) was dissolved in dichloromethane (20 ml) and diluted with pyridine (233 mg, 3.0 mmol). Methanesulfonyl chloride (300 mg, 2.60 mmol) was added dropwise. After 16 hours, the reaction was diluted with water and $CHCl_3$. The layers were separated and the aqueous layer was extracted with $CHCl_3$ (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. recrystallization (EtOH/H_2O) yielded 206 mg of white crystals (29%). ^{1}H-NMR (200MHz, $CDCl_3$): δ 1.10-1.40 (m, 4H), 1.45 (s, 9H), 2.00-2.20 (m, 4H), 2.98 (s, 3H), 3.20-3.50 (brs, 2H), 4.37 (brs, 1H); MS (ES) 293.1 (M^++1), 278.1 (M^+-15), 237.1 (M^+-56).

4-(4-*trans*-methanesulfamidocyclohexyl)amino-5,6-dimethyl-2-phenyl-7H-(1-phenylethyl)pyrrolo[2,3d]pyrimidine.

4-(N-sulfonyl)-N-*tert*-butoxycarbonyl-*trans*-1,4-cyclohexyldiamine (206 mg, 0.71 mmol), was dissolved in dichloromethane (5ml) and diluted with TFA (6 ml). After 16

hours, the reaction was concentrated. The crude reaction mixture, DMSO (2 ml), $NaHCO_3$ (100 mg, 1.1 mmol) and 1-chloro-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine were combined in a flask and heated to 130 °C. After 15 hours, the reaction was cooled to room temperature, and diluted with EtOAc (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Chromatography (silica preparatory plate, 20:1 $CHCl_3$/EtOH) yielded 2.6 mg of a tan solid (5% yield). MS (ES): 414.2 (M^++1).

Example 1:

A solution of 4-chloro-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (0.50 g, 1.94 mmol) and 4-*trans*-hydroxycyclohexylamine (2.23 g, 19.4 mmol) in methyl sulfoxide (10.0 mL) was heated at 130°C for 5 hr. After cooling down to room temperature, water (10.0 mL) was added and the resulted aqueous solution was extracted with EtOAc (3 x10.0 mL). The combined EtOAc solution was dried ($MgSO_4$) and filtered, the filtrate was concentrated *in vacuo* to dryness, the residue was chromatographed on silica gel to give 0.49 g (75%) of 4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. mp 197-199°C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25-1.59 (m, 8H), 2.08 (s, 3H), 2.29 (s, 3H), 3.68-3.79 (m, 1H), 4.32-4.38 (m, 1H), 4.88 (d, J = 8 Hz, 1H), 7.26-7.49 (m, 3H), 8.40-8.44 (dd, J = 2.2, 8 Hz, 2H), 10.60 (s, 1H); MS (ES): 337.2 (M^++1).

The following compounds were obtained in a similar manner to that of Example 1:

4-(4-*trans*-hydroxycyclohexyl)amino-6-methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, CDCl3) δ 11.37 (s, 1H, pyrrole-NH), 8.45 (m, 2H, Ar-H), 7.55 (m, 3H, Ar-H), 6.17 (s, 1H, pyrrole-H), 4.90 (br d, 1H, NH), 4.18 (m, 1H, CH-O), 3.69 (m, 1H, CH-N), 2.40-2.20 (m, 2H), 2.19-1.98 (m, 2H), 2.25 (s, 3H, CH3) 1.68-1.20 (m, 4H); MS (ES): 323.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5-methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, CDCl3) δ 11.37 (s, 1H, pyrrole-NH), 8.40 (m, 2H, Ar-H), 7.45 (m, 3H, Ar-H), 5.96 (s, 1H, pyrrole-H), 4.90 (br d, 1H, NH), 4.18 (m, 1H, CH-O), 3.69 (m, 1H, CH-N), 2.38-2.20 (m, 2H), 2.18-1.98 (m, 2H), 2.00 (s, 3H, CH3) 1.68-1.20 (m, 4H); MS (ES): 323.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. mp 245.5-246.5°C; ^{1}H NMR (200MHz, CD_3OD) δ 8.33(m, 2H, Ar-H), 7.42 (m, 3H, Ar-H), 7.02 (d, 1H, J=3.6 Hz, pyrolle-H), 6.53 (d, 1H, J=3.6 Hz, pyrolle-H), 4.26 (m, 1H, CH-O), 3.62 (m, 1H, CH-N), 2.30-2.12 (m, 2H), 2.12-1.96 (m, 2H), 1.64-1.34 (m, 4H); MS, M+1=309.3; Anal ($C_{18}H_{20}N_4O$) C, H, N.

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(3-pyridyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.21-1.54 (m, 8H); 2.28 (s, 3H); 2.33 (s, 3H); 3.70 (m, 1H), 4.31(m, 1H), 4.89 (d, 1H), 7.40 (m, 1H), 8.61 (m, 2H), 9.64 (m, 1H); MS (ES): 338.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(2-furyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26-1.64(m, 8H), 2.22 (s, 3H), 2.30 (s, 3H), 3.72(m, 1H), 4.23 (m, 1H), 4.85 (d, 1H), 6.52(m, 1H), 7.12 (m, 1H), 7.53 (m, 1H), 9.28 (s, 1H); MS (ES): 327.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(3-furyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25-1.63 (m, 8 H), 2.11 (s, 3H), 2.27 (s, 3H), 3.71(m, 1H), 4.20 (m, 1H), 4.84 (d, 1H), 7.03 (m, 1H), 7.45(m, 1H), 8.13(m, 1H), 10.38 (m, 1H); MS (ES): 327.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-cyclopentyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26-2.04 (m, 16 H), 2.26 (s, 3H), 2.27 (s, 3H), 3.15(m, 1H), 3.70 (m, 1H), 4.12 (m, 1H), 4.75(d, 1H); MS (ES): 329.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(2-thienyl)-7*H*-pyrrolo[2,3d]pyrimidin-4-amine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28-1.59 (m, 8H), 2.19 (s, 3H), 2.29 (s, 3H), 3.74 (m, 1H), 4.19 (m, 1H), 4.84 (d, 1H), 7.09 (m, 1H), 7.34 (m, 1H), 7.85 (m, 1H), 9.02 (s, 1H); MS (ES): 343.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(3-thienyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.21-1.60 (m, 8H), 1.98 (s, 3H), 2.23 (s, 3H), 3.66 (m, 1H), 4.22 (m, 1H), 7.27 (m, 1H), 7.86 (m, 1H), 8.09 (m, 1H), 11.23 (s, 1H); MS (ES): 343.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(4-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26- 1.66 (m, 8H), 1.94 (s, 3H), 2.28 (s, 3H), 3.73 (m, 1H), 4.33 (m, 1H), 4.92 (d, 1H), 7.13 (m, 2H), 8.41 (m, 2H), 11.14 (s, 1H); MS (ES): 355.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(3-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26-1.71 (m, 8H), 2.06 (s, 3H), 2.30 (s, 3H), 3.72 (m, 1H), 4.30 (m, 1H), 4.90 (d, 1H), 7.09 (m, 1H), 7.39 (m, 1H), 8.05 (m, 1H), 8.20 (m, 1H), 10.04 (s. 1H); MS (ES): 355.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(2-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30-1.64 (m, 8H), 2.17 (s, 3H), 2.31 (s, 3H), 3.73 (m, 1H), 4.24 (m, 1H), 4.82 (d, 1H), 7.28 (m, 2H), 8.18 (m, 1H), 9.02 (m, 1H), 12.20 (s, 1H); MS (ES): 355.3 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-isopropyl-7*H*-pyrrolo[2,3d]pyrimidine ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.31 (d, J = 7.0 Hz, 6H), 1.30-1.65 (m, 8H), 2.27 (s, 3H), 2.28 (s, 3H), 3.01 (m, J = 7.0 Hz, 1H), 3.71 (m, 1H), 4.14 (m, 1H), 4.78 (d, 1H); MS (ES): 303.2.

dl-4-(2-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-isopropyl-7*H*-pyrrolo[2,3d]pyrimidine ^{1}H NMR (200 MHz, $CDCl_3$) d 1.31-1.42 (br, 4H), 1.75-1.82 (br, 4H), 2.02 (s, 3H), 2.29 (s, 3H), 3.53 (m, 1H), 4.02 (m, 1H), 5.08 (d, 1H), 7.41-7.48 (m, 3H), 8.30 (m, 2H), 10.08 (s, 1H); MS (ES): 337.2 (M^++1).

4-(3,4-*trans*-dihydroxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 353.2 (M^++1).

4-(3,4-*cis*-dihydroxylcyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 353.2 (M^++1).

4-(2-acetylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. mp 196-199°C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.72 (s, 3H), 1.97 (s, 3H), 2.31 (s, 3H), 3.59 (m, 2H), 3.96 (m, 2H), 5.63 (br, 1H), 7.44-7.47 (m, 3H), 8.36-8.43 (dd, J = 1 Hz, 7 Hz, 2H), 10.76 (s, 1H); MS (ES): 324.5 (M^++1).

dl-4-(2-*trans*-hydroxycyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.[1]

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.62 (m, 2H), 1.79 (br, 4H), 1.92 (s, 3H), 2.29 (s, 3H), 4.11 (m, 1H), 4.23 (m, 1H), 5.28 (d, 1H), 7.41-7.49 (m, 3H), 8.22 (m, 2H), 10.51 (s, 1H); MS (ES): 323.2 (M^++1).

[1] For preparation of 2-*trans*-hydroxycyclopentylamine, see PCT 9417090.

dl-4-(3-*trans*-hydroxycyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.[1]

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.58-1.90 (br, 6 H,), 2.05 (s, 3H), 2.29 (s, 3H), 4.48-4.57 (m, 1H), 4.91-5.01 (m, 2H), 7.35-7.46 (m, 3H), 8.42-8.47 (m, 2H), 10.11 (s, 1H); MS (ES): 323.2 (M^++1).

[1] For preparation of 3-*trans*-hydroxycyclopentylamine, see EP-A-322242.

dl-4-(3-*cis*-hydroxycyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.[1]

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.82-2.28 (br, 6H), 2.02 (s, 3H), 2.30 (s, 3H), 4.53-4.60 (m, 1H), 4.95-5.08 (m, 1H), 5.85-5.93 (d, 1H), 7.35-7.47 (m, 3H), 8.42-8.46 (m, 2H), 10.05 (s, 1H); MS (ES): 323.2 (M^++1).

[1] For preparation of 3-*cis*-hydroxycyclopentylamine, see EP-A-322242.

4-(3,4-*trans*-dihydroxycyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.[1] ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.92-1.99 (br, 2H), 2.14 (s, 3H), 2.20 (br, 2H), 2.30 (s, 3H), 2.41-2.52 (br, 2H), 4.35 (m, 2H), 4.98 (m, 2H), 7.38-7.47 (m, 3H), 8.38-8.42 (m, 2H), 9.53 (s, 1H); MS (ES): 339.2 (M^++1).

[1] For preparation of 3,4-*trans*-dihydroxycyclopentylamine, see PCT 9417090.

4-(3-amino-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.29 (s, 3H), 2.71 (t, 2H), 4.18 (m, 2H), 5.75-5.95 (m, 3H), 7.38-7.48 (m, 3H), 8.37-8.41 (m, 2H), 10.42 (s, 1H); MS (ES): 310.1 (M^++1).

4-(3-N-cyclopropylmethylamino-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, CD_3OD) δ 0.51 (q, 2H), 0.40 (q, 2H), 1.79-1.95 (br, 1H), 2.36 (s, 3H), 2.40 (s, 3H), 2.72 (t, 2H), 2.99 (d, 2H), 4.04 (t, 2H), 7.58-7.62 (m, 3H), 8.22-8.29 (m, 2H); MS (ES): 364.2 (M^++1).

4-(2-amino-2-oxoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine ^1H NMR (200 MHz, CD_3OD) δ 2.31 (s, 3H), 2.38 (s, 3H), 4.26 (s, 2H), 7.36 (m, 3H), 8.33 (m, 2H); MS (ES): 396.1 (M^++1).

4-(2-N-methylamino-2-oxoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^1H NMR (200 MHz, $CDCl_3$) δ 1.99 (s, 3H), 2.17 (s, 3H), 2.82 (d, 3H), 4.39 (d, 2H), 5.76 (t, 1H), 6.71 (br, 1H), 7.41-7.48 (m, 3H), 8.40 (m, 2H), 10.66 (s, 1H); MS (ES): 310.1 (M^++1).

4-(3-*tert*-butyloxyl-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^1H NMR (200 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.96 (s, 3H), 2.29 (s, 3H), 2.71 (t, 2H), 4.01 (q, 2H), 5.78 (t, 1H), 7.41-7.48 (m, 3H), 8.22-8.29 (m, 2H); MS (ES): 367.2 (M^++1).

4-(2-hydroxyethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^1H NMR (200 MHz, $CDCl_3$) δ 1.92 (s, 3H), 2.29 (s, 3H), 3.81-3.98 (br, 4H), 5.59 (t, 1H), 7.39-7.48 (m, 3H), 8.37 (m, 2H), 10.72 (s, 1H); MS (ES): 283.1 (M^++1).

4-(3-hydroxypropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^1H NMR (200 MHz, $CDCl_3$) δ 1.84 (m, 2H), 1.99 (s, 3H), 2.32 (s, 3H), 3.62 (t, 2H), 3.96 (m, 2H), 3.35 (t, 1H), 7.39-7.48 (m, 3H), 8.36 (m, 2H), 10.27 (s, 1H); MS (ES): 297.2 (M^++1).

4-(4-hydroxybutyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^1H NMR (200 MHz, $CDCl_3$) δ 1.71-1.82 (m, 4H), 1.99 (s, 3H), 2.31 (s, 3H), 3.68-3.80 (m, 4H), 5.20 (t, 1H), 7.41-7.49 (m, 3H), 8.41(m, 2H), 10.37 (s, 1H); MS (ES): 311.2 (M^++1).

4-(4-*trans*-acetylaminocyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

4-(4-*trans*-methylsulfonylaminocyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

4-(2-acetylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidine.

4-(4-*trans*-hydoxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-1-phenylethyl)pyrrolo[2,3d]pyrimidine.

4-(3-pyridylmethyl)amino-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidine.

4-(2-methylpropyl)amino-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidine.

Example 2:

To a stirred suspension of triphenylphosphine (0.047 g, 0.179 mmol) and benzoic acid (0.022 g, 0.179 mmol) in THF (1.0 mL) cooled to 0°C was added 4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (0.05 g, 0.149 mmol) at 0°C. Diethyl azodicarboxylate (0.028 ml, 0.179 mmol) was then added dropwise over 10 minutes. The reaction was then allowed to warm to room temperature. After reaction was complete by TLC the reaction mixture was quenched with aqueous sodium bicarbonate (3.0 mL). The aqueous phase was separated and extracted with ether (2 X 5.0 mL). The organic extracts were combined, dried, and concentrated *in vacuo* to dryness. To the residue was added ether (2.0 mL) and hexane (5.0 mL) whereupon the bulk of the triphenylphosphine oxide was filtered off. Concentration of the filtrate gave a viscous oil which was purified by column chromatography (hexane:ethyl acetate=4:1) to give 5.0 mg (7.6%) of 4-(4-*cis*-benzoyloxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 441.3 (M^{+}+1). The reaction also produced 50.0 mg (84%) of 4-(3-

cyclohexenyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 319.2 (M^{+}+1).

Example 3:

To a solution of 4-(4-*cis*-benzoyloxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (5.0 mg, 0.0114 mmol) in ethanol (1.0 mL) was added 10 drops of 2M sodium hydroxide. After 1 hr, the reaction mixture was extracted with ethyl acetate (3 x 5.0 mL) and the organic layer was dried, filtered and concentrated *in vacuo* to dryness. The residue was subjected to column chromatography (hexane:ethyl acetate=4:1) to give 3.6 mg (94 %) of 4-(4-*cis*-hydroxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 337.2 (M^{+}+1).

The following compounds were obtained in a similar manner as that of Example 3:

4-(3-N,N-dimethyl-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.01 (s, 3H), 2.31 (s, 3H), 2.73 (t, 2H), 2.97 (s, 6H), 4.08 (m, 2H), 6.09 (t, 1H), 7.41-7.48 (m, 3H), 8.43 (m, 2H), 10.46 (s, 1H); MS (ES): 338.2 (M^{+}+1).

4-(2-formylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.26 (s, 3H), 2.37 (s, 3H), 3.59-3.78 (m, 2H), 3.88-4.01 (m, 2H), 5.48-5.60 (m, 1H), 7.38-7.57 (m, 3H), 8.09 (s, 1H), 8.30-8.45 (m, 2H), 8.82 (s, 1H); MS (ES): 310.1 (M^{+}+1).

4-(3-acetylaminopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 338.2 (M^{+}+1).

Example 4:

4-(3-*tert*-butyloxy-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (70.0 mg, 0.191 mmol)) was dissolved in trifluoroacetic acid:dichloromethane (1:1, 5.0 mL). The resulting solution was stirred at room temperature for 1 hr. and then refluxed for 2 hr. After cooling down to room

temperature, the mixture was concentrated *in vacuo* to dryness. The residue was subjected to preparative thin layer chromatography (EtOAc:hexane:AcOH=7:2.5:0.5) to give 40.0 mg (68%) of. 4-(3-hydroxy-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, CD_3OD) δ 2.32 (s, 3H), 2.38 (s, 3H), 2.81 (t, 2H), 4.01 (t, 2H), 7.55 (m, 3H), 8.24 (m, 2H); MS (ES): 311.1 (M^++1).

The following compound was obtained in a similar manner as that of Example 4:

4-(3-aminopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 296.1 (M^++1), 279.1 (M^+-NH_3).

Example 5:

4-(3-hydroxy-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (50.0 mg, 0.161 mmol) was dissolved in a mixture of N,N-dimethylformamide (0.50 mL), dioxane (0.50 mL) and water(0.25 mL). To this solution was added methylamine (0.02 mL, 40% wt in water, 0.242 mmol), triethylamine (0.085 mL) and N,N,N'N'-tetramethyl uronium tetrafluoroborate (61.2 mg, 0.203 mmol). After stirring at room temperature for 10 min, the solution was concentrated and the residue was subjected to preparative thin layer chromatography (EtOAc) to give 35.0 mg (67%) of 4-(3-N-methyl-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.92 (s, 3H), 2.30 (s, 3H), 2.65 (t, 2H), 4.08 (t, 2H), 5.90 (t, 1H), 6.12 (m, 1H), 7.45 (m, 3H), 8.41 (m, 2H), 10.68 (s, 1H); MS (ES): 311.1 (M^++1).

The following compounds were obtained in a similar manner as that of Example 5:

4-(2-cyclopropanecarbonylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 350.2 (M^++1).

4-(2-isobutyrylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 352.2 (M^++1).

4-(3-propionylaminopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00-1.08 (t, 3H), 1.71-2.03 (m, 4H), 2.08 (s, 3H), 2.37 (s, 3H), 3.26-3.40 (m, 2H), 3.79-3.96 (m, 2H), 5.53-5.62 (m, 1H), 6.17-6.33 (m, 1H), 7.33-7.57 (m, 3H), 8.31-8.39 (m, 2H), 9.69 (s, 1H); MS (ES): 352.2 (M^++1).

4-(2-methylsulfonylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.18 (s, 3H), 2.27 (s, 3H), 2.92 (s, 3H), 3.39-3.53 (m, 2H), 3.71-3.88 (m, 2H), 5.31-5.39 (m, 1H), 6.17-6.33 (m, 1H), 7.36-7.43 (m, 3H), 8.20-8.25 (m, 2H), 9.52 (s, 1H); MS (ES): 360.2 (M^++1).

Example 6:

A mixture of 4-chloro-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine (0.70 g, 2.72 mmol) and 1,2-diaminoethane (10.0 mL, 150 mmol) was refluxed under inert atmosphere for 6 hr. The excess amine was removed *in vacuo*, the residue was washed sequentially with ether and hexane to give 0.75 g (98%) of 4-(2-aminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES); 282.2 (M^++1), 265.1 (M^+-NH_3).

Example 7:

To a solution of 4-(2-aminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (70.0 mg, 0.249 mmol) and triethylamine (50.4 mg, 0.498 mmol) in dichloromethane (2.0 mL) was added propionyl chloride (25.6 mg, 0.024 mL, 0.274 mmol) at 0°C. After 1 hr, the mixture was concentrated *in vacuo* and the residue was subjected to preparative thin layer chromatography (EtOAc) to give 22.0 mg (26%) of 4-(2-propionylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine MS (ES): 338.2 (M^++1).

The following compounds were obtained in a similar manner as that of Example 7:

4-(2-N'-methylureaethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.13 (s, 3H), 2.32 (s, 3H), 3.53 (d, 3H), 3.55 (m, 2H), 3.88 (m, 2H), 4.29 (m, 1H), 5.68 (t, 1H), 5.84 (m, 1H), 7.42 (m, 3H), 8.36 (dd, 2H), 9.52 (s, 1H); MS (ES): 339.3 (M^++1).

4-(2-N'-ethylureaethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 353.2 (M^++1).

Example 8:

To a solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (41.1 mg, 0.215 mmol), dimethylaminopyridine (2.4 mg, 0.020 mmol) and pyruvic acid (18.9 mg, 0.015 mL, 0.215 mmol) in dichloromethane (2.0 mL) was added 4-(2-aminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (55.0 mg, 0.196 mmol). The mixture was stirred at room temperature for 4 hr. Usual workup and column chromatography (EtOAc) then gave 10.0 mg (15%) of 4-(2'-pyruvylamidoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.. MS (ES): 352.2 (M^++1).

Example 9:

To a solution of 4-(2-aminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (60.0 mg, 0.213 mmol) in dichloromethane (2.0 mL) was added N-trimethylsilyl isocyanate (43.3 mg, 0.051 mL, 0.320 mmol). The mixture was stirred at room temperature for 3 hr followed by addition of aqueous sodium bicarbonate. After filtration through small amount of silica gel, the filtrate was concentrated *in vacuo* to dryness to give 9.8 mg (14%) of 4-(2-ureaethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 325.2 (M^++1).

The following compounds were obtained in a similar manner as that of Example 9:

dl-4-(2-acetylaminopropyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28–1.32 (d, J=8 Hz, 3 H), 1.66 (s, 3H), 1.96 (s, 3H), 2.30 (s, 3H) 3.76-3.83 (m, 2H), 4.10-4.30 (m, 1H), 5.60-5.66 (t, J=6 Hz, 1H), 7.40-7.51(m, 3H), 8.36-8.43 (m, 2H), 10.83 (s, 1H); MS (ES): 338.2 (M^++1).

(R)-4-(2-acetylaminopropyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.31 (d, 3H), 1.66 (s, 3H) 1.99 (s, 3H), 2.31 (s, 3H), 3.78-3.83 (m, 2H), 4.17-4.22 (m, 1H), 5.67 (t, 1H), 7.38-7.5 (m, 3H), 8.39 (m, 2H), 10.81 (s, 1H); MS (ES): 338.2 (M^++1).

(R)-4-(1-methyl-2-acetylaminoethyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (d, 3H), 1.68 (s, 3H), 2.21 (s, 3H), 2.34 (s, 3H), 3.46-3.52 (br, m, 2H), 4.73 (m, 1H), 5.22 (d, 1H), 7.41-7.46 (m, 3H), 8.36-8.40 (m, 2H), 8.93 (s, 1H); MS (ES): 338.2 (M^++1).

(S)-4-(2-acetylaminopropyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.31 (d, 3H), 1.66 (s, 3H) 2.26 (s, 3H), 2.35 (s, 3H), 3.78-3.83 (m, 2H), 4.17-4.22 (m, 1H), 5.67 (t, 1H), 7.38-7.5 (m, 3H), 8.39 (m, 2H), 8.67(s, 1H); MS (ES): 338.2 (M^++1).

(S)-4-(1-methyl-2-acetylaminoethyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (d, 3H), 1.68 (s, 3H), 2.05 (s, 3H), 2.32 (s, 3H), 3.46-3.52 (m, 2H), 4.73 (m, 1H), 5.22 (d, 1H), 7.41-7.46 (m, 3H), 8.36-8.40 (m, 2H), 10.13 (s, 1H); MS (ES): 338.2 (M^++1).

Example 10:

Reaction of 4-chloro-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine with the mixture of *dl*-1-amino-2-(1,1-dimethylethoxy)carbonylamino-propane and *dl*-2-amino-1-(1,1-dimethylethoxy)carbonylamino-propane was run in a similar manner as that of Example 1. The reaction gave a mixture of *dl*-4-(1-methyl-2-(1,1-

dimethylethoxy)carbonylamino)ethylamino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine and *dl*-4-(2-methyl-2-(1,1-dimethylethoxy)carbonylamino)ethylamino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine which were separated by column chromatography (EtOAc:hexanes=1:3). The first fraction was *dl*-4-(1-methyl-2-(1,1-dimethylethoxy)carbonylaminoethyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.29 – 1.38 (m, 12 H), 1.95 (s, 3H), 2.31 (s, 3H) 3.34-3.43 (m, 2H), 4.62-4.70 (m, 1H), 5.36-5.40 (d, J=8 Hz, 1H), 5.53 (br, 1H), 7.37-7.49(m, 3H), 8.37-8.44(m, 2H), 10.75 (s, 1H). MS 396.3 (M^++1); The second fraction was *dl*-4-(2-(1,1-dimethylethoxy)carbonylaminopropyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26–1.40 (m, 12 H), 2.00 (s, 3H), 2.31 (s, 3H) 3.60-3.90 (m, 2H), 3.95-4.10 (m, 1H), 5.41-5.44 (d, J=6.0 Hz, 1H), 5.65(br, 1H), 7.40-7.46(m, 3H), 8.37-8.44(m, 2H), 10.89 (s, 1H); MS (ES): 396.2 (M^++1).

The following compounds were obtained in a similar manner as that of <u>Example 10:</u>

(S,S)-4-(2-acetylaminocyclohexyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.43 (m, 4 H), 1.60 (s, 3 H), 1.83 (m, 2 H), 2.18 (s, 3 H), 2.30 (m, 2 H), 2.32 (s, 3 H), 3.73 (br, 1H), 4.25 (br, 1H), 5.29 (d, 1H), 7.43-7.48 (m, 3H), 8.35-8.40 (m, 2H), 9.05 (s, 1 H).

4-(2-methyl-2-acetylaminopropyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.51 (s, 6H), 1.56 (s, 3H), 2.07 (s, 3H), 2.36 (s, 3H), 3.76 (d, 2H), 5.78 (t, 1H), 7.41-7.48 (m, 3H), 7.93 (s, 1H), 8.39 (m, 2H), 10.07 (s, 1H); MS (ES): 352.3 (M^++1).

<u>Example 11:</u>

dl-4-(1-methyl-2-(1,1-dimethylethoxy)carbonylaminoethyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine (60.6 mg, 0.153 mmol) was treated with trifluoroacetic acid (0.5 mL) in dichloromethane (2.0 mL) for 14 hr. The organic solvent

was removed *in vacuo* to dryness. The residue was dissolved in N,N-dimethylformamide (2.0 mL) and triethylamine (2.0 mL). To the solution at 0°C was added acetic anhydride (17.2 mg, 0.016, 0.169 mmol). The resulted mixture was stirred at room temperature for 48 hr and then concentrated *in vacuo* to dryness. The residue was subjected to preparative thin layer chromatography (EtOAc) to give 27.0 mg (52%) of *dl*-4-(1-methyl-2-acetylaminoethyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.38–1.42 (d, J=8 Hz, 3 H), 1.69 (s, 3H), 2.01 (s, 3H), 2.32 (s, 3H) 3.38-3.60 (m, 2H), 4.65-4.80 (m, 1H), 5.23-5.26 (d, J=6 Hz, 1H), 7.40-7.51(m, 3H), 8.37-8.43(m, 2H), 10.44 (s, 1H); MS (ES): 338.2 (M^++1).

Example 12:

(R,R)-4-(2-aminocyclohexyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine, prepared in a similar manner as that of Example 1 from 4-chloro-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (0.15 g, 0.583 mmol) and (1R, 2R)-(-)-1,2-diaminocyclohexane (0.63 g, 5.517 mmol), was treated with triethylamine (0.726 g, 7.175 mmol) and acetic anhydride (0.325 g, 3.18 mmol) in N,N-dimethylformamide (10.0 mL) at room temperature for 2 hr. After removal of solvent *in vacuo*, ethyl acetate (10.0 mL) and water (10.0 mL) were added to the residue. The mixture was separated and the aqueous layer was extracted with ethyl acetate (2 x 10.0 mL). The combined ethyl acetate solution was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo to dryness and the residue was subjected to column chromatography (EtOAc:Hexane=1:1) to give 57.0 mg (26%) of (R,R)-4-(2-acetylaminocyclohexyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.43 (m, 4 H), 1.60 (s, 3 H), 1.84 (m, 2 H), 2.22 (s, 3 H), 2.30 (m, 2 H), 2.33 (s, 3 H), 3.72 (br, 1H), 4.24 (br, 1H), 5.29 (d, 1H), 7.43-7.48 (m, 3H), 8.35-8.39 (m, 2H), 8.83 (s, 1 H); MS (ES): 378.3 (M^++1).

Example 13:

To a solution of 4-(2-hydroxyethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (40.0 mg, 0.141 mmol) in pyridine (1.0 mL) was added acetic anhydride (0.108 g, 1.06 mmol) at 0°C. The mixture was stirred at room temperature for

4 hr and the solvent was removed in vacuo. The residue was subjected to preparative thin layer chromatography (EtOAc:hexane=1:1) to give 32.3 mg (71%) of 4-(2-acetyloxyethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.90 (s, 3H), 2.08 (s, 3H), 2.31 (s, 3H), 4.05 (m, 2H), 4.45 (t, 2H), 5.42 (m, 1H), 7.41-7.49 (m, 3H), 8.42(m, 2H), 11.23 (s, 1H).

Example 14:

A solution of Fmoc-β-Ala-OH (97.4 mg, 0.313 mmol) and oxalyl chloride (39.7 mg, 27.3 μL, 0.313 mmol) in dichloromethane (4.0 mL) with 1 drop of N,N-dimethylformamide was stirred at 0°C for 1 hr followed by addition of 4-(2-aminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (80.0 mg, 0.285 mmol) and triethylamine (57.6 mg, 79.4 μL, 0.570 mmol) at 0°C. After 3 hr, the mixture was concentrated *in vacuo* and the residue was treated with the solution of 20% piperidine in N,N-dimethylforamide (2.0 mL) for 0.5 hr. After removal of the solvent *in vacuo,* the residue was washed with diethyl ether:hexane (1:5) to give 3.0 mg (3%) of 4-(6-amino-3-aza-4-oxohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 353.2 (M^++1).

Example 15:

A solution of 4-(2-aminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (70.0 mg, 0.249 mmol) and succinic anhydride (27.0 mg, 0.274 mmol) in dichloromethane (4.0 mL) with 1 drop of N,N-dimethylformamide was stirred at room temperature for 4 hr. The reaction mixture was extracted with 20% sodium hydroxide (3 x 5.0 mL). The aqueous solution was acidified with 3 M hydrochloride to pH = 7.0. The whole mixture was extracted with ethyl acetate (3 x 10 mL). The combined organic solution was dried ($MgSO_4$) and filtered. The filtrate was concentrated *in vacuo* to dryness to give 15.0 mg (16%) of 4-(7-hydroxy-3-aza-4,7-dioxoheptyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 382.2 (M^++1).

Example 16:

To 10 mL of dimethylformamide (DMF) at room temperature were added 700 mg of 4-*cis*-3-hydroxycyclopentyl)amino-2-phenyl-5,6-dimethyl-7*H*-pyrrolo[2,3d]pyrimidine followed by 455 mg of N-Boc glycine, 20 mg of N,N-dimethylaminopyridine (DMAP), 293 mg of hydroxybenzotriazole (HOBT) and 622 mg of 1-(3-dimethylaminopropyl)-3-ethylcarboiimide hydrochloride (EDCl). The reaction mixture was left stirring overnight. DMF was then removed under reduced pressure and the reaction mixture was partitioned between 20mL of ethyl acetate and 50mL of water. The aqueous portion was extracted further with 2x20mL of ethyl acetate and the combined organic portions were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. Purification on silica gel, eluting with ethyl acetate/hexane gave 410 mg of the desired product: 4-(*cis*-3-(N-t-butoxycarbonyl-2-aminoacetoxy) cyclopentyl) amino-2-phenyl-5,6,-dimethyl-7*H*-pyrrolo[2,3d]pyrimidine, MS (ES) (M^{+}+1)=480.2. The ester was then treated with 5 mL of 20% trifluoroacetic acid in dichloromethane at room temperature, left over night and then concentrated. Trituration with ethyl acetate gave 300 mg of an off white solid; 4-(*cis*-3-(2-aminoacetoxy)cyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine trifluoroacetic acid salt, MS (ES) (M^{+}+1)=380.1.

One skilled in the art will appreciate that the following compounds can be synthesized by the methods disclosed above:

4-(*cis*-3-hydroxycyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine MS (ES) (M^{+}+1)= 323.1.

4-(*cis*-3-(2-aminoacetoxy)cyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidinetrifluoroacetic acid salt MS (ES) (M^{+}+1)= 380.1.

4-(3-acetamido)piperidinyl-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine MS (ES) (M^{+}+1)= 364.2.

4-(2-N'-methylureapropyl)amino-5,6-dimethyl-2-phenyl-*7H*-pyrrolo[2,3d] pyrimidine, MS (ES) (M^{+}+1)=353.4.

4-(2-acetamidobutyl)amino-5,6-dimethyl-2-phenyl-*7H*-pyrrolo[2,3d]pyrimidine, MS (ES) (M^{+}+1)= 352.4.

4-(2-N'-methylureabutyl)amino-5,6-dimethyl-2-phenyl-*7H*-pyrrolo[2,3d]pyrimidine MS (ES) (M^{+}+1)= 367.5

4-(2-aminocyclopropylacetamidoethyl)amino-2-phenyl-*7H*-pyrrolo[2,3d] pyrimidine MS (ES) (M^{+}+1)= 309.1.

4-(*trans*-4-hydroxycyclohexyl)amino-2-(3-chlorophenyl)-*7H*-pyrrolo[2,3d] pyrimidine MS (ES) (M^{+}+1)=342.8.

4-(*trans*-4-hydroxycyclohexyl)amino-2-(3-fluorophenyl)-*7H*-pyrrolo [2,3d] pyrimidine MS (ES) (M^{+}+1)=327.2.

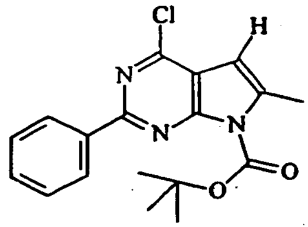
4-(*trans*-4-hydroxycyclohexyl)amino-2-(4-pyridyl)-*7H*-pyrrolo[2,3d]pyrimidine MS (ES) (M^{+}+1)=310.2.

Example 17

Scheme IX

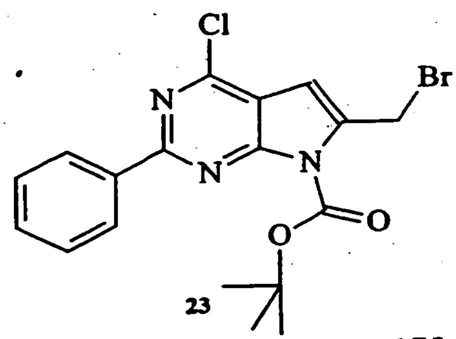
The pyrrole nitrogen of (7) (Scheme IX) was protected with di-*t*-butyldicarbonate under basic conditions to yield the corresponding carbamate (22). Radical bromination of (22) proceeded regioselectively to yield bromide (23). In general, compound (23) served as a key electrophilic intermediate for various nucleophilic coupling partners. Displacement of the alkyl bromide with sodium phenolate trihydrate yielded compound (24). Subsequent displacement of the aryl chloride and removal of the t-butyl carbamate protecting group occurred in one step yielding desired compound (25).

Detailed Synthesis of Compounds (22)-(25) in Accordance with Scheme IX



22

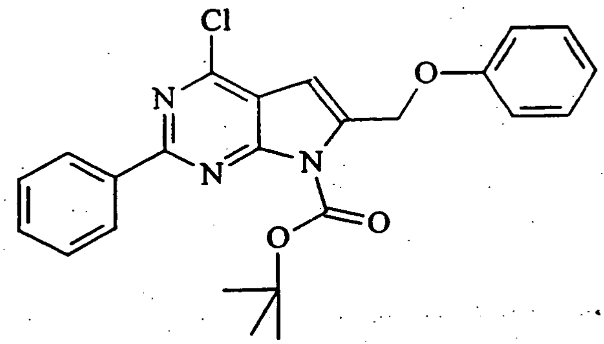
Di-*t*-butyl dicarbonate (5.37 g, 24.6 mmol) and dimethylaminopyridine (1.13 g, 9.2 mmol) were added to a solution containing (7) (1.50 g, 6.15 mmol) and pyridine (30 mL). After 20 h the reaction was concentrated and the residue was partitioned between CH_2Cl_2 and water. The CH_2Cl_2 layer was separated, dried over $MgSO_4$, filtered and concentrated to yield a black solid. Flash chromatography (SiO_2; 1/9 EtOAc/Hexanes, R_f0.40) yielded 1.70 g (80%) of a white solid (22). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.50 (m, 2H, Ar-H), 7.45 (m, 3H, Ar-H), 6.39 (s, 1H, pyrrole-H), 2.66 (s, 3H,pyrrole-*CH3*),



23

1.76 (s, 9H, carbamate-*CH3*); MS, M + 1 = 344.1; Mpt = 175-177°C.

N-Bromosuccinimide (508 mg, 2.86 mmol) and AIBN (112 mg, 0.68 mmol) were added to a solution containing (22) (935 mg, 2.71 mmol) and CCl_4 (50 mL). The solution was heated to reflux. After 2 h the reaction was cooled to room temperature and concentrated *in vacuo* to yield a white solid. Flash chromatography (SiO_2; 1/1 CH_2Cl_2/Hexanes, R_f0.30) yielded 960 mg (84%)of a white solid (23). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.52 (m, 2H, Ar-H), 7.48 (m, 3H, Ar-H), 6.76 (s, 1H, pyrrole-H), 4.93 (s, 2H,pyrrole-*CH2*Br), 1.79 (s, 9H, carbamate-*CH3*); MS, M + 1 = 423.9; Mpt = 155-157°C.



24

Sodium phenoxide trihydrate (173 mg, 1.02 mmol) was added in one portion to a solution of bromide (23) (410 mg, 0.97 mmol) dissolved in CH_2Cl_2 (5 mL) and DMF (10 mL). After 2 h the reaction solution was partitioned between CH_2Cl_2 and water. The water layer was extracted with CH_2Cl_2. The combined CH_2Cl_2 layers were washed with water, dried over $MgSO_4$, filtered and concentrated to yield a yellow solid. Flash chromatography (SiO_2; 1/6 EtOAc/Hexanes, *Rf* 0.30) yielded 210 mg (50%) of a white solid (24). ^1H NMR (200 MHz, $CDCl_3$) δ 8.53 (m, 2H, Ar-H), 7.48 (m, 3H, Ar-H), 7.34 (m, 2H, Ar-H), 7.03 (m, 3H, Ar-H), 6.83 (s, 1H, pyrrole-H), 5.45 (s, 2H, $ArCH_2O$), 1.76 (s, 9H, carbamate-CH_3); MS, M^+ = 436.2.

25

A solution containing (24) (85 mg, 0.20 mmol), N-acetylethylenediamine (201 mg, 1.95 mmol) and DMSO (3 mL) was heated to 100°C. After 1 h the temperature was raised to 130°C. After 3 h the reaction was cooled to room temperature and partitioned between EtOAc and water. The water layer was extracted with EtOAc (2x). The combined EtOAc layers are washed with water, dried over $MgSO_4$, filtered and concentrated. Flash chromatography (SiO_2; 1/10 EtOH/$CHCl_3$, *Rf* 0.25) yielded 73 mg (93%)of a white foamy solid (25). ^1H NMR (200 MHz, DMSO-d6) δ 11.81 (br s, 1H, N-H), 8.39 (m, 2H, Ar-H), 8.03 (br t, 1H, N-H), 7.57 (br t, 1H, N-H), 7.20 – 7.50 (m, 5H, Ar-H), 6.89 – 7.09 (m, 3H, Ar-H), 6.59 (s, 1H, pyrrole-H), 5.12 (s, 2H, ArCH_2O), 3.61 (m, 2H, NCH_2), 3.36 (m, 2H, NCH_2), 1.79 (s, 3H,COCH_3); MS, M+ 1 = 402.6

The following compounds were obtained in a manner similar to that of Example 17:

4-(2-acetylaminoethyl)amino-6-phenoxymethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. mp 196-197 °C; MS (ES): 401.6 (M^{+}+1).

4-(2-acetylaminoethyl)amino-6-(4-fluorophenoxy)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS(ES): 420.1 (M^{+}+1).

4-(2-acetylaminoethyl)amino-6-(4-chlorophenoxy)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS(ES): 436.1 (M^{+}+1).

4-(2-acetylaminoethyl)amino-6-(4-methoxyphenoxy)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS(ES): 432.1 (M^{+}+1).

4-(2-acetylaminoethyl)amino-6-(N-pyridin-2-one)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS(ES): 403.1 (M^{+}+1).

4-(2-acetylaminoethyl)amino-6-(N-phenylamino)methyl-2-phenyl-7*H*-pyrrolo[2,3]pyrimidine. MS(ES): 400.9 (M^{+}+1).

4-(2-acetylaminoethyl)amino-6-(N-methyl-N-phenylamino)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS(ES): 414.8 (M^{+}+1).

4-(2-N'-methylureaethyl)amino-6-phenoxymethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 416.9 (M^{+}+1).

Yeast β-Galactosidase reporter gene assays for human adenosine A1 and A2a receptor

Yeast strains (*S. cerevisiae*) were transformed with human adenosine A1 (A1R; CADUS strain CY12660) or human A2a (A2a; CADUS strain CY8362) and the addition of a lacZ(β-Galactosidase) reporter gene to utilize as a functional readout. A complete description of the transformations is listed below (see Yeast Strains). NECA (5'-N-

ethylcarboxamidoadenosine), a potent adenosine receptor agonist with similar affinity for A1 and A2a receptors, was used as a ligand for all assays. Test compounds were examined at 8 concentrations (0.1 – 10,000 nM) for ability to inhibit NECA-induced β-Galactosidase activity by CY12660 or CY8362.

__Preparation of Yeast Stock Cultures__. Each of the respective yeast strains, CY12660 and CY8362, were streaked onto an LT agar plate and incubated at 30°C until colonies were observed. Yeast from these colonies were added to LT liquid (pH 6.8) and grown overnight at 30°C. Each yeast strain was then diluted to an OD_{600} = 1.0-2.0 (approximately 1-2 X 10^7 cells/ml), as determined spectrophotometrically (Molecular Devices VMAX). For each 6 ml of yeast liquid culture, 4 ml of 40% glycerol (1:1.5 vol:vol) was added ("yeast/glycerol stock"). From this yeast/glycerol stock, ten 1 ml aliquots were prepared and stored at -80°C until required for assay.

__Yeast A1R and A2aR Assay__. One vial each of CY8362 and CY12660 yeast/glycerol stock was thawed and used to inoculate Supplemented LT liquid media, pH 6.8 (92 ml LT liquid, to which is added: 5 ml of 40% glucose, 0.45 ml of 1M KOH and 2.5 ml of Pipes, pH 6.8). Liquid cultures were grown 16-18 hr (overnight) at 30°C. Aliquots from overnight cultures were then diluted in LT media, containing 4U/ml adenosine deaminase (Type VI or VII from calf intestinal mucosa, Sigma), to obtain OD_{600} = 0.15 (1.5 X 10^6 cells/ml) for CY8362 (A2aR) and OD_{600} = 0.50 (5X10^6 cells/ml) for CY12660 (A1R).

Assays were conducted with a final volume of 100 ul in 96-well microtiter plates, such that a final concentration of 2% DMSO was achieved in all wells. For primary screening, 1-2 concentrations of test compounds were utilized (10 uM, 1μM). For compound profiling, 8 concentrations were tested (10000, 1000, 500, 100, 50, 10, 1 and 0.1 nM). To each microtiter plate, 10 ul of 20% DMSO was added to "Control" and "Total" wells while 10 ul of Test Compound (in 20% DMSO) was added to "Unknown" wells. Subsequently, 10 ul of NECA (5 uM for A1R, 1 uM for A2aR) were added to "Total" and "Unknown" wells; 10 ul of PBS was added to the "Control" wells. In the

final addition, 80 ul of yeast strain, CY8362 or CY12660, were added to all wells. All plates were then agitated briefly (LabLine orbital shaker 2-3 min) and allowed to incubate for 4 hrs. at $30^{O}C$ in a dry oven.

β-Galactosidase activity can be quantitated using either colorimetric (*e.g.*, ONPG, CPRG), luminescent (*e.g.*, Galacton-Star) or fluorometric substrates (*e.g.*, FDG Resorufin) substrates. Currently, fluorescence detection is preferred on the basis of superior signal:noise ratio, relative freedom from interference and low cost. Fluoresceii digalactopyranoside (FDG, Molecular Probes or Marker Gene Technologies), a fluorescent β-Galactosidase substrate, was added to all wells at 20 ul/well (final concentration = 80 uM). Plates were shaken for 5-6 sec (LabLine orbital shaker) and then incubated at $37^{O}C$ for 90 min (95% O_2/5% CO_2 incubator). At the end of the 90 min incubation period, β-Galactosidase activity was stopped using 20 ul/well of 1M Na_2CO_3 and all plates shaken for 5-6 sec. Plates were then agitated for 6 sec and relativ fluorescence intensity determined using a fluorometer (Tecan Spectrafluor; excitation = 485 nm, emission = 535 nm).

Calculations. Relative fluorescence values for "Control" wells were interpreted as background and subtracted from "Total" and "Unknown" values. Compound profiles were analyzed via logarithmic transformation (x-axis: compound concentration) followed by one site competition curve fitting to calculate IC_{50} values (GraphPad Prism).

Yeast strains. Saccharomyces cerevisiae strains CY12660 [far1*1442 tbt1-1 fus1-HIS3 can1 ste14::trp1::LYS2 ste3*1156 gpa1(41)-Gαi3 lys2 ura3 leu2 trp1: his3; LEU2 PGKp-Mfα1Leader-hA1R-PHO5term 2mu-orig REP3 Ampr] and CY8362 [gpa1p-rGαsE10K far1*1442 tbt1-1 fus1-HIS3 can1 ste14::trp1: LYS2 ste3*1156 lys2 ura3 leu2 trp1 his3; LEU2 PGKp-hA2aR 2mu-ori REP3 Ampr] were developed.

LT Media. LT (Leu-Trp supplemented) media is composed of 100g DIFCO yeast nitrogen base, supplemented with the following: 1.0g valine, 1.0g aspartic acid, 0.75g phenylalanine, 0.9g lysine, 0.45g tyrosine, 0.45g isoleucine, 0.3g methionine, 0.6g adenine, 0.4g uracil, 0.3g serine, 0.3g proline, 0.3g cysteine, 0.3g arginine, 0.9g histidin and 1.0g threonine.

Construction of Yeast Strains Expressing Human A1 Adenosine Receptor

In this example, the construction of yeast strains expressing a human A1 adenosine receptor functionally integrated into the yeast pheromone system pathway is described.

I. Expression Vector Construction

To construct a yeast expression vector for the human A1 adenosine receptor, the A1 adenosine receptor cDNA was obtained by reverse transcriptase PCR of human hippocampus mRNA using primers designed based on the published sequence of the human A1 adenosine receptor and standard techniques. The PCR product was subcloned into the *Nco*I and *Xba*I sites of the yeast expression plasmid pMP15.

The pMP15 plasmid was created from pLPXt as follows: The *Xba*I site of YEP51 (Broach, J.R. *et al.* (1983) "Vectors for high-level, inducible expression of cloned genes in yeast" p. 83-117 *in* M. Inouye (ed.), Experimental Manipulation of Gen Expression. Academic Press, New York) was eliminated by digestion, end-fill and religation to create Yep51*Nco*D*Xba*. Another *Xba*I site was created at the *BamHI* site b digestion with *BamHI*, end-fill, linker (New England Biolabs, # 1081) ligation, *Xba*I digestion and re-ligation to generate YEP51*Nco*Xt. This plasmid was digested with *Esp*31 and *Nco*I and ligated to Leu2 and PGKp fragments generated by PCR. The 2 kb Leu2 PCR product was generated by amplification from YEP51*Nco* using primers containing *Esp*31 and *Bgl*II sites. The 660 base pair PGKp PCR product was generated by amplification from pPGKαs (Kang, Y.-S. *et al.* (1990) *Mol. Cell. Biol.* 10:2582-2590) with PCR primers containing *Bgl*II and *Nco*I sites. The resulting plasmid is calle pLPXt. pLPXt was modified by inserting the coding region of the a-factor pre-pro leader into the *Nco*I site. The prepro leader was inserted so that the *Nco*I cloning site

was maintained at the 3' end of the leader, but not regenerated at the 5' end. In this way receptors can be cloned by digestion of the plasmid with *Nco*I and *Xba*I. The resulting plasmid is called pMP15.

The pMP15 plasmid into which was inserted the human A1 adenosine receptor cDNA was designated p5095. In this vector, the receptor cDNA is fused to the 3' end of the yeast a-factor prepro leader. During protein maturation the prepro peptide sequences are cleaved to generate mature full-length receptor. This occurs during processing of the receptor through the yeast secretory pathway. This plasmid is maintained by Leu selection (i.e., growth on medium lacking leucine). The sequence of the cloned coding region was determined and found to be equivalent to that in the published literature (GenBank accession numbers S45235 and S56143).

II. Yeast Strain Construction

To create a yeast strain expressing the human A1 adenosine receptor, yeast strain CY7967 was used as the starting parental strain. The genotype of CY7967 is as follows: *MATα gpaD1163 gpa1(41)Gαi3 far1D1442 tbt-1 FUS1-HIS3 can1 ste14::trp1::LYS2 ste3D1156 lys2 ura3 leu2 trp1 his3*

The genetic markers are reviewed below:

MATa......................	Mating type a.
gpa1D1163...........	The endogenous yeast G-protein GPA1 has been deleted.
gpa1(41)Gαi3........	gpa1(41)-Gai3 was integrated into the yeast genome. This chimeric Ga protein is composed of the first 41 amino acids of the endogenous yeast Ga subunit GPA1 fused to the mammalian G-protein Gai3 in which the cognate N-terminal amino acids have been deleted.
far1D1442............	FAR1 gene (responsible for cell cycle arrest) has been deleted (thereby preventing cell cycle arrest upon activation of the pheromone response pathway).
tbt-1.....................	strain with high transformation efficiency by electroporation.

FUS1-HIS3.........	a fusion between the FUS1 promoter and the HIS3 coding region (thereby creating a pheromone inducible HIS3 gene).
can 1....................	arginine/canavinine permease.
ste14::trp1::LYS2....	gene disruption of STE14, a C-farnesyl methyltransferase (thereby lowering basal signaling through the pheromone pathway).
ste3D1156............	endogenous yeast STR, the a factor pheromone receptor (STE3) was disrupted.
lys2....................	defect in 2-aminoapidate reductase, yeast need lysine to grow.
ura3....................	defect in orotidine-5'-phosphate decarboxylase, yeast need uracil to grow
leu2....................	defect in b-isopropylmalate dehydrogenase, yeast need leucine to grow.
trp1....................	defect in phosphoribosylanthranilate, yeast need tryptophan to grow.
his3....................	defect in imidazoleglycerolphosphate dehydrogenase, yeast need histidine to grow.

Two plasmids were transformed into strain CY7967 by electroporation: plasmid p5095 (encoding human A1 adenosine receptor; described above) and plasmid p1584, which is a FUS1-β-galactosidase reporter gene plasmid. Plasmid p1584 was derived from plasmid pRS426 (Christianson, T.W. *et al.* (1992) *Gene* 110:119-1122). Plasmid pRS426 contains a polylinker site at nucleotides 2004-2016. A fusion between the FUS1 promoter and the β-galactosidase gene was inserted at the restriction sites *Eag*I and *Xho*I to create plasmid p1584. The p1584 plasmid is maintained by Trp selection (i.e., growth on medium lacking leucine).

The resultant strain carrying p5095 and p1584, referred to as CY12660, expresses the human A1 adenosine receptor. To grow this strain in liquid or on agar plates, minimal media lacking leucine and tryptophan was used. To perform a growth assay on plates (assaying *FUS1-HIS3*), the plates were at pH 6.8 and contained 0.5-2.5 mM 3-amino-1,2,4-triazole and lacked leucine, tryptophan and histidine. As a control for specificity, a comparison with one or more other yeast-based seven transmembrane receptor screens was included in all experiments.

Construction of Yeast Strains Expressing Human A2a Adenosine Receptor

In this example, the construction of yeast strains expressing a human A2a adenosine receptor functionally integrated into the yeast pheromone system pathway is described.

I. Expression Vector Construction

To construct a yeast expression vector for the human A2a adenosine receptor, the human A2a receptor cDNA was obtained from Dr. Phil Murphy (NIH). Upon receipt of this clone, the A2a receptor insert was sequenced and found to be identical to the published sequence (GenBank accession # S46950). The receptor cDNA was excised from the plasmid by PCR with VENT polymerase and cloned into the plasmid pLPBX, which drives receptor expression by a constitutive Phosphoglycerate Kinase (PGK) promoter in yeast. The sequence of the entire insert was once again sequenced and found to be identical with the published sequence. However, by virtue of the cloning strategy employed there were three amino acids appended to the carboxy-terminus of the receptor, GlySerVal.

II. Yeast Strain Construction

To create a yeast strain expressing the human A2a adenosine receptor, yeast strain CY8342 was used as the starting parental strain. The genotype of CY8342 is as follows:

MATa far1D1442 tbt1-1 lys2 ura3 leu2 trp1 his3 fus1-HIS3 can1 ste3D1156 gpaD1163 ste14::trp1::LYS2 gpa1p-rG$_{\alpha s}$E10K (or *gpa1p-rG$_{\alpha s}$D229S* or *gpa1p-rG$_{\alpha s}$E10K+D229S*)

The genetic markers are as described in Example 1, except for the G-protein variation. For human A2a receptor-expression, yeast strains were utilized in which the endogenous yeast G protein GPA1 had been deleted and replaced by a mammalian $G_{\alpha s}$. Three rat $G_{\alpha s}$ mutants were utilized. These variants contain one or two point mutations which convert them into proteins which couple efficiently to yeast $\beta\gamma$. They are identified as $G_{\alpha s}$E10K (in which the glutamic acid at position ten is replaced with lysine), $G_{\alpha s}$D229S

(in which the aspartic acid at position 229 is replaced with serine) and $G_{\alpha s}$E10K+D229S (which contains both point mutations).

Strain CY8342 (carrying one of the three mutant rat $G_{\alpha s}$ proteins) was transformed with either the parental vector pLPBX (Receptor^{-}) or with pLPBX-A2a (Receptor^{+}). A plasmid with the FUS1 promoter fused to β-galactosidase coding sequences (described in above) was added to assess the magnitude of activation of the pheromone response pathway.

Functional Assay using Yeast Strains Expressing Human A1 Adenosine Receptor

In this example, the development of a functional screening assay in yeast for modulators of the human A1 adenosine receptor is described.

I. Ligands Used in Assay

Adenosine, a natural agonist for this receptor, as well as two other synthetic agonists were utilized for development of this assay. Adenosine, reported to have an EC_{50} of approximately 75 nM, and (-)-N6-(2-phenylisopropyl)-adenosine (PIA) with a reported affinity of approximately 50 nM were used in a subset of experiments. 5'-N-ethylcarboxamido-adenosine (NECA) was used in all growth assays. To prevent signaling due to the presence of adenosine in the growth media, adenosine deaminase (4U/ml) was added to all assays.

II. Biological Response in Yeast

The ability of the A1 adenosine receptor to functionally couple in a heterologous yeast system was assessed by introducing the A1 receptor expression vector (p5095, described above) into a series of yeast strains that expressed different G protein subunits The majority of these transformants expressed G_{α} subunits of the $G_{\alpha i}$ or $G_{\alpha o}$ subtype. Additional G_{α} proteins were also tested for the possible identification of promiscuous receptor-Gα protein coupling. In various strains, a *STE18* or a chimeric *STE18*-Gγ2 construct was integrated into the genome of the yeast. The yeast strains harbored a defective *HIS3* gene and an integrated copy of *FUS1-HIS3*, thereby allowing for

selection in selective media containing 3-amino-1,2,4-triazole (tested at 0.2, 0.5 and 1.0 mM) and lacking histidine. Transformants were isolated and monolayers were prepared on media containing 3-amino-1,2,4-triazole, 4 U/ml adenosine deaminase and lacking histidine. Five microliters of various concentrations of ligand (*e.g.*, NECA at 0, 0.1, 1.0 and 10 mM) was applied. Growth was monitored for 2 days. Ligand-dependent growth responses were tested in this manner in the various yeast strains. The results are summarized in Table 1 below. The symbol (-) indicates that ligand-dependent receptor activation was not detected while (+) denotes ligand-dependent response. The term "LIRMA" indicates ligand independent receptor mediated activation.

Table 3

Yeast strain	Gα subunit	Gγ subunit	Strain Variants	Result
CY1316	GPA1	STE18		-
	GPA41-$G_{\alpha i1}$			+
	GPA41-$G_{\alpha i2}$			+
	GPA41-$G_{\alpha i3}$			+
	GPA41-$G_{\alpha i2}$-$G_{\alpha OB}$			LIRMA
	GPA41-$G_{\alpha SE10K}$			-
	GPA41-$G_{\alpha SD229S}$			-
CY7967	GPA41-$G_{\alpha i3}$-integrated	STE18		+++
CY2120	GPA1	STE18	sst2Δ	+
	GPA41-$G_{\alpha i1}$			+
	GPA41-$G_{\alpha i2}$			+
	GPA41-$G_{\alpha i3}$			+
	GPA41-$G_{\alpha i2}$-$G_{\alpha OB}$			LIRMA
	GPA41-$G_{\alpha SE10K}$			-
	GPA41-$G_{\alpha SD229S}$			-
CY9438	GPA1	STE18-Gγ2		-
	GPA41-$G_{\alpha i1}$			+
	GPA41-$G_{\alpha i2}$			+
	GPA41-$G_{\alpha i3}$			+
	GPA41-$G_{\alpha i2}$-$G_{\alpha OB}$			LIRMA
	GPA41-$G_{\alpha SE10K}$			-
	GPA41-$G_{\alpha SD229S}$			-
CY10560	GPA1-integrated	STE18-Gγ2	sst2Δ	++

As indicated in Table 3, the most robust signaling was found to occur in a yeast strain expressing the GPA1(41)-$G_{\alpha i3}$ chimera.

III. *fus1*-LacZ Assay

To characterize activation of the pheromone response pathway more fully, synthesis of β-galactosidase through *fus1*LacZ in response to agonist stimulation was measured. To perform the β-galactosidase assay, increasing concentrations of ligand were added to mid-log culture of human A1 adenosine receptor expressed in a yeast strain co-expressing a Ste18-Gγ2 chimera and GPA_{41}-$G_{\alpha i3}$. Transformants were isolated and grown overnight in the presence of histidine and 4 U/ml adenosine deaminase. After five hours of incubation with 4 U/ml adenosine deaminase and ligand, induction of β-galactosidase was measured using CPRG as the substrate for β-galactoside. 5×10^5 cells were used per assay.

The results obtained with NECA stimulation indicated that at a NECA concentration of 10^{-8} M approximately 2-fold stimulation of β-galactosidase activity was achieved. Moreover, a stimulation index of approximately 10-fold was observed at a NECA concentration of 10^{-5} M.

The utility of this assay was extended by validation of the activity of antagonists on this strain. Two known adenosine antagonist, XAC and DPCPX, were tested for their ability to compete against NECA (at 5 mM) for activity in the β-galactosidase assay. In these assays, β-galactosidase induction was measured using FDG as the substrate and 1.6×10^5 cells per assay. The results indicated that both XAC and DPCPX served as potent antagonists of yeast-expressed A1 adenosine receptor, with IC_{50} values of 44 nM and 49 nM, respectively.

In order to determine if this inhibitory effect was specific to the A1 subtype, a series of complementary experiments were performed with the yeast-based A2a recepto assay (described in Example 4). Results obtained with the A2a yeast-based assay indicated that XAC was a relatively effective A2a receptor antagonist, consistent with published reports. In contrast, DPCPX was relatively inert at this receptor, as expected from published reports.

IV. Radioligand Binding

The A1 adenosine receptor assay was further characterized by measurement of the receptor's radioligand binding parameters. Displacement binding of [^{3}H]CPX by several adenosine receptor reference compounds, XAC, DPCPX, and CGS, was analyzed using membranes prepared from yeast expressing the human A1 adenosine receptor. The results with yeast membranes expressing the human A1 adenosine receptor were compared to those from yeast membranes expressing the human A2a adenosine receptor or the human A3 receptor to examine the specificity of binding. To perform the assay, fifty mg of membranes were incubated with 0.4 nM [^{3}H]CPX and increasing concentrations of adenosine receptor ligands. Incubation was in 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 10 mM $MgCl_2$, 0.25 % BSA and 2 U/ml adenosine deaminase in the presence of protease inhibitors for 60 minutes at room temperature. Binding was terminated by addition of ice-cold 50 mM Tris-HCl, pH 7.4 plus 10 mM $MgCl_2$, followed by rapid filtration over GF/B filters previously soaked with 0.5 % polyethyenimine, using a Packard 96-well harvester. Data were analyzed by nonlinear least square curve fitting procedure using Prism 2.01 software. The IC_{50} values obtained in this experiment are summarized in Table 4, below:

Table 4

		IC_{50} [nM]	
Compound	hA1R	hA2aR	hA3R
XAC	6.6	11.7	53.1
DPCPX	8.5	326.4	1307.0
CGS-15943	13.1	15.8	55.5
NECA	215.5	294.9	34.9
R-PIA	67.6	678.1	23.6
IB-MECA	727.7	859.4	3.1
Alloxozine	1072.0	1934.0	8216.0

These data indicate that the reference compounds have affinities consistent with those reported in the literature. The data further indicate that the yeast-based assays are of sufficient sensitivity to discriminate receptor subtype specificity.

Functional Assay using Yeast Strains Expressing Human A2a Adenosine Receptor

In this example, the development of a functional screening assay in yeast for modulators of the human A1 adenosine receptor is described.

I. Ligands Used in Assay

The natural ligand adenosine, as well as other thoroughly characterized and commercially available ligands were used for study of the human A2a receptor functionally expressed in yeast. Three ligands have been used in the establishment of this assay. They include:

Ligand	Reported K_i	Function
Adenosine	500 nM	agonist
5'-N-ethylcarboxamidoadenosine (NECA)	10-15 nM	agonist
(-)-N6-(2-phenylisopropyl)-adenosine (PIA)	100-125 nM	agonist

To prevent signaling due to the presence of adenosine in the growth media, adenosine deaminase (4U/ml) was added to all assays.

II. Biological Response in Yeast

A2a receptor agonists were tested for the capacity to stimulate the pheromone response pathway in yeast transformed with the A2a receptor expression plasmid and expressing either $G_{\alpha s}$E10K, $G_{\alpha s}$D229S or $G_{\alpha s}$E10K+D229S. The ability of ligand to stimulate the pheromone response pathway in a receptor dependent manner was indicated by an alteration in the yeast phenotype. Receptor activation modified the phenotype from histidine auxotrophy to histidine prototrophy (activation of *fus1-HIS3*). Three independent transformants were isolated and grown overnight in the presence of

histidine. Cells were washed to remove histidine and diluted to 2×10^6 cells/ml. 5 µl o each transformant was spotted onto nonselective media (including histidine) or selective media (1 mM AT) in the absence or presence of 4 U/ml adenosine deaminase. Plates were grown at 30 °C for 24 hours. In the presence of histidine both Receptor$^+$ (R^+) and Receptor$^-$ (R^-) strains were capable of growth. However, in the absence of histidine only R^+ cells grew. Since no ligand had been added to these plates two explanations were possible for this result. One possible interpretation was that the receptor bearing yeast were at a growth advantage due to Ligand Independent Receptor Mediated Activation (LIRMA). Alternatively the yeast could have been synthesizing the ligand adenosine. To distinguish between these two possibilities, an enzyme which degrades the ligand, adenosine deaminase (ADA), was added to the growing yeast and plates. In the presence of adenosine deaminase R^+ cells no longer grew in the absence of histidine indicating that the yeast were indeed synthesizing ligand.

This interpretation was confirmed by an A2a growth assay in liquid. In this experiment R^+ yeast (a $G_{\alpha s}$E10K strain expressing the A2a receptor) were inoculated a three densities (1×10^6 cell/ml; 3×10^5 cells/ml; or 1×10^5 cells/ml) in the presence or absence of adenosine deaminase (4 U/ml). The stringency of the assay was enhanced with increasing concentrations (0, 0.1, 0.2 or 0.4 mM)of 3-amino-1,2,4-triazole (AT), a competitive antagonist of imidazoleglycerol-P dehydratase, the protein product of the HIS3 gene. In the presence of adenosine deaminase and 3-amino-1,2,4-triazole yeast grew less vigorously. However in the absence of 3-amino-1,2,4-triazole, adenosine deaminase had little effect. Thus adenosine deaminase itself had no direct effect upon the pheromone response pathway.

An alternative approach to measuring growth and one that can be miniaturized for high throughput screening is an A2a receptor ligand spot assay. A $G_{\alpha s}$E10K strain expressing the A2a receptor (A2aR+) or lacking the receptor (R-) was grown overnight in the presence of histidine and 4 U/ml adenosine deaminase. Cells were washed to remove histidine and diluted to 5×10^6 cells/ml. 1×10^6 cells were spread onto selective plates containing 4 U/ml adenosine deaminase and 0.5 or 1.0 mM 3-amino-1,2,4-triazole (AT) and allowed to dry for 1 hour. 5 µl of the following reagents were

applied to the monolayer: 10 mM adenosine, 38.7 mM histidine, dimethylsulfoxide (DMSO), 10 mM PIA or 10 mM NECA. Cells were grown 24 hours at 30 °C. The results showed that cells without receptor could only grow when histidine was added to the media. In contrast, R^+ cells only grew in areas where the A2a receptor ligands PIA and NECA had been spotted. Since the plates contained adenosine deaminase, the lack of growth where adenosine had been spotted confirmed that adenosine deaminase was active.

III. *fus1* LacZ Assay

To quantitate activation of the yeast mating pathway, synthesis of β-galactosidase through *fus1*LacZ was measured. Yeast strains expressing $G_{\alpha s}$E10K, $G_{\alpha s}$D229S or $G_{\alpha s}$E10K+D229S were transformed with a plasmid encoding the human A2a receptor (R+) or with a plasmid lacking the receptor (R-). Transformants were isolated and grown overnight in the presence of histidine and 4 U/ml adenosine deaminase. 1×10^7 cells were diluted to 1×10^6 cells/ml and exposed to increasing concentrations of NECA for 4 hours, followed by determination of the β-galactosidase activity in the cells. The results demonstrated that essentially no β-galactosidase activity was detected in R- strains, whereas increasing amounts of β-galactosidase activity were detected in R+ strains expressing either $G_{\alpha s}$E10K, $G_{\alpha s}$D229S or $G_{\alpha s}$E10K+D229S as the concentration of NECA increased, indicating a dose dependent increase in units of β-galactosidase detected in response to exposure to increased ligand concentration. This dose dependency was only observed in cells expressing the A2a receptor. Furthermore the most potent $G_{\alpha s}$ construct for the A2a receptor was $G_{\alpha s}$E10K. The $G_{\alpha s}$D229S construct was the second-most potent $G_{\alpha s}$ construct for the A2a receptor, while the $G_{\alpha s}$E10K+D229S construct was the least potent of the three $G_{\alpha s}$ constructs tested, although even the $G_{\alpha s}$E10K+D229S construct stimulated readily detectable amounts of β-galactosidase activity.

For a further description of the assays identified, see U.S. Application Serial Nc 09/088985, entitled "Functional Expression of Adenosine Receptors in Yeast", filed June 2, 1998 (Attorney Docket No. CPI-093), the entire contents of which are hereby incorporated herein by reference.

Pharmacological Characterization of the Human Adenosine Receptor Subtypes

Material and Methods

Materials. [^{3}H]-DPCPX [Cyclopentyl-1,3-dipropylxantine, 8-[dipropyl-2,3-^{3}H(N)] (120.0 Ci/mmol); [^{3}H]-CGS 21680, [carboxyethyl-^{3}H (N)] (30 Ci/mmol) and [^{125}I] – AB-MECA ([^{125}I]–4-Aminobenzyl-5'-N-Methylcarboxamideoadenosine) (2,200 Ci/mmol) were purchased from New England Nuclear (Boston, MA). XAC (Xantine amine congener); NECA (5'-N-Ethylcarboxamidoadenosine); and IB-MECA from Research Biochemicals International (RBI, Natick, MA). The Adenosine Deaminase ar Complete protease inhibitor cocktail tablets were purchased from Boehringer Mannhei Corp. (Indianapolis, IN). Membranes from HEK-293 cells stably expressing the humar Adenosine 2a [RB-HA2a]; Adenosine 2b [RB-HA2b] or Adenosine 3 [RB-HA3] receptor subtypes, respectively were purchased from Receptor Biology (Beltsville, MI Cell culture reagents were from Life Technologies (Grand Island, NY) except for serui that was from Hyclone (Logan, UT).

Yeast strains. Saccharomyces cerevisiae strains CY12660 [far1*1442 tbt1-1 fus1-HIS can1 ste14::trp1::LYS2 ste3*1156 gpa1(41)-Gαi3 lys2 ura3 leu2 trp1: his3; LEU2 PGKp-Mfα1Leader-hA1R-PHO5term 2mu-orig REP3 Ampr] and CY8362 [gpa1p-rC sE10K far1*1442 tbt1-1 fus1-HIS3 can1 ste14::trp1: LYS2 ste3*1156 lys2 ura3 leu2 trp1 his3; LEU2 PGKp-hA2aR 2mu-ori REP3 Ampr] were developed as described above.

Yeast culture. Transformed yeast were grown in Leu-Trp [LT] media (pH 5.4) supplemented with 2% glucose. For the preparation of membranes 250 ml of LT medium were inoculated with start titer of 1-2 x 10^6 cells/ml from a 30 ml overnight

culture and incubated at 30^{O}C under permanent oxygenation by rotation. After 16 h growth the cells were harvested by centrifugation and membranes were prepared as described below.

Mammalian Tissue Culture. The HEK-293 cells stably expressed human Adenosine 2a receptor subtype (Cadus clone # 5) were grown in Dulbeco's minimal essential media (DMEM) supplemented with 10% fetal bovine serum and 1X penicillin/streptomycin under selective pressure using 500 mg/ml G418 antibiotic, at 37^{O}C in a humidified 5% CO_2 atmosphere.

Yeast Cell Membrane Preparations. 250 ml cultures were harvested after overnight incubation by centrifugation at 2,000 x g in a Sorvall RT6000 centrifuge. Cells were washed in ice-cold water, centrifuged at 4^{O}C and the pellet was resuspended in 10 ml ice-cold lysis buffer [5 mM Tris-HCl, pH 7.5; 5 mM EDTA; and 5 mM EGTA] supplemented with Protease inhibitor cocktail tablets (1 tablet per 25 ml buffer). Glass beads (17 g; Mesh 400-600; Sigma) were added to the suspension and the cells were broken by vigorous vortexing at 4^{O}C for 5 min. The homogenate was diluted with additional 30 ml lysis buffer plus protease inhibitors and centrifuged at 3,000 x g for 5 min. Subsequently the membranes were peleted at 36,000 x g (Sorvall RC5B, type SS34 rotor) for 45 min. The resulting membrane pellet was resuspended in 5 ml membrane buffer [50 mM Tris-HCl, pH 7.5; 0.6 mM EDTA; and 5 mM $MgCl_2$] supplemented wit Protease inhibitor cocktail tablets (1 tablet per 50 ml buffer) and stored at -80 ^{O}C for further experiments.

Mammalian Cell Membrane Preparations. HEK-293 cell membranes were prepared a described previously (*Duzic E et al.: J. Biol. Chem., 267, 9844-9851, 1992*) Briefly, cells were washed with PBS and harvested with a rubber policeman. Cells were pelted a 4^{O}C 200 x g in a Sorvall RT6000 centrifuge. The pellet was resuspended in 5 ml/dish o lysis buffer at 4^{O}C (5 mM Tris-HCl, pH 7.5; 5 mM EDTA; 5 mM EGTA; 0.1 mM Phenylmethylsulfonyl fluoride, 10 mg/ml pepstatin A; and 10 mg/ml aprotinin) and homogenized in a Dounce homogenizer. The cell lysate was then centrifuged at 36,000

x g (Sorvall RC5B, type SS34 rotor) for 45 min and the pellet resuspended in 5 ml membrane buffer [50 mM Tris-HCl, pH 7.5; 0.6 mM EDTA; 5 mM $MgCl_2$; 0.1 mM Phenylmethylsulfonyl fluoride, 10 mg/ml pepstatin A; and 10 mg/ml aprotinin) and stored at -80 ^{o}C for further experiments.

The Bio-Rad protein assay kits, based on the Bradford dye-binding procedure, (Bradford, M.: *Anal. Biochem.* 72:248 (1976)) were used to determine total protein concentration in yeast and mammalian membranes.

Adenosine 1 receptor subtype saturation and competition radioligand binding.

Saturation and competition binding on membranes from yeast cell transformed with human A1 receptor subtype were carried out using antagonist [^{3}H] DPCPX as a radioactive ligand. Membranes was diluted in binding buffer [50 mM Tris-HCl, pH 7.4; containing 10 mM $MgCl_2$; 1.0 mM EDTA; 0.25% BSA; 2 U/ml adenosine deaminase and 1 protease inhibitor cocktail tablet/50 ml] at concentrations of 1.0 mg/ml.

In saturation binding membranes (50 μg/well) were incubate with increasing concentrations of [^{3}H] DPCPX (0.05 - 25 nM) in a final volume of 100 μl of binding buffer at 25^{o}C for 1 hr in the absence and presence of 10 μM unlabeled XAC in a 96-well microtiter plate.

In competition binding membranes (50 μg/well) were incubate with [^{3}H] DPCPX (1.0 nM) in a final volume of 100 ml of binding buffer at 25^{o}C for 1 hr in the absence and presence of 10 μM unlabeled XAC or increasing concentrations of competing compounds in a 96-well microtiter plate.

Adenosine 2a receptor subtype competition radioligand binding

Competition binding on membranes from HEK293 cell stably expressing the human A2a receptor subtype were carried out using agonist [^{3}H] CGS-21680 as a radioactive ligand. Membranes was diluted in binding buffer [50 mM Tris-HCl, pH 7.4; containing 10 mM $MgCl_2$; 1.0 mM EDTA; 0.25% BSA; 2 U/ml adenosine deaminase and 1 protease inhibitor cocktail tablet/50 ml] at concentrations of 0.2 mg/ml.

Membranes (10 μg/well) were incubate with [^{3}H] CGS-21680 (100 nM) in a final volume of 100 ml of binding buffer at 25^{o}C for 1 hr in the absence and presence of 50 μM unlabeled NECA or increasing concentrations of competing compounds in a 96-well microtiter plate.

Adenosine 3 receptor competition radioligand binding

Competition binding on membranes from HEK293 cell stably expressing the human A3 receptor subtype were carried out using agonist [^{125}I] AB-MECA as a radioactive ligand. Membranes was diluted in binding buffer [50 mM Tris-HCl, pH 7.4; containing 10 mM $MgCl_2$; 1.0 mM EDTA; 0.25% BSA; 2 U/ml adenosine deaminase and 1 protease inhibitor cocktail tablet/50 ml] at concentrations of 0.2 mg/ml. Membranes (10 μg/well) were incubate with [^{125}I] AB-MECA (0.75 nM) in a final volume of 100 μl of binding buffer at 25^{o}C for 1 hr in the absence and presence of 10 μM unlabeled IB-MECA or increasing concentrations of competing compounds in a 96-well microtiter plate.

At the end of the incubation, the A1, A2a and A3 receptor subtypes radioligand binding assays was terminated by the addition of ice-cold 50 mM Tris-HCl (pH 7.4) buffer supplemented with 10 mM $MgCl_2$, followed by rapid filtration over glass fiber filters (96-well GF/B UniFilters, Packard) previously presoaked in 0.5% polyethylenimine in a Filtermate 196 cell harvester (Packard). The filter plates were dried coated with 50 μl /well scintillation fluid (MicroScint-20, Packard) and counted in a TopCount (Packard). Assays were performed in triplicate. Non-specific binding was 5.6 ± 0.5%, 10.8 ± 1.4% and 15.1 ± 2.6% of the total binding in a A1R, A2aR and A3R binding assay, respectively.

Adenosine 2b receptor subtype competition radioligand binding

Competition binding on membranes from HEK293 cell stably expressing the human A2b receptor subtype were carried out using A1 receptor antagonist [^{3}H] DPCPX as a radioactive ligand. Membranes was diluted in binding buffer [10 mM Hepes-KOH, pH 7.4; containing 1.0 mM EDTA; 0.1 mM Benzamidine and 2 U/ml

adenosine deaminase] at concentrations of 0.3 mg/ml. Membranes (15 μg/well) were incubate with [^{3}H] DPCPX (15 nM) in a final volume of 100 μl of binding buffer at 25^{o}C for 1 hr in the absence and presence of 10 μM unlabeled XAC or increasing concentrations of competing compounds in a 96-well microtiter plate. At the end of the incubation, the assay was terminated by the addition of ice-cold 10 mM Hepes-KOH (pH 7.4) buffer followed by rapid filtration over glass fiber filters (96-well GF/C UniFilters, Packard) previously presoaked in 0.5% polyethylenimine in a Filtermate 196 cell harvester (Packard). The filter plates were dried coated with 50 μl/well scintillation fluid (MicroScint-20, Packard) and counted in a TopCount (Packard). Assays were performed in triplicate. Non-specific binding was 14.3 ± 2.3% of the total binding.

Specific binding of [^{3}H] DPCPX; [^{3}H] CGS-21680 and [^{125}I] AB-MECA was defined as the difference between the total binding and non-specific binding. Percent inhibition of the compounds was calculated against total binding. Competition data were analyzed by iterative curve fitting to a one site model, and K_I values were calculated from IC_{50} values (Cheng and Prusof, Biochem. Pharmacol. 22, 3099-3109, 1973) using the GraphPad *Prizm 2.01* software.

Results

A primary function of certain cell surface receptors is to recognize appropriate ligands. Accordingly, we determined ligand binding affinities to establish the functional integrity of the Adenosine 1 receptor subtype expressed in yeast. Crude membranes prepared from *Saccharomyces cerevisiae* transformed with human Adenosine 1 receptor subtype construct exhibited specific saturable binding of [^{3}H] DPCPX with a K_D of 4.0 ± 0.19 nM. The K_D and B_{max} value were calculated from the saturation isotherm and Scatchard transformation of the data indicated a single class of binding sites. The densities of adenosine binding sites in the yeast membrane preparations were estimated to 716.8 ± 43.4 fmol/mg membrane protein.

The pharmacological subtype characteristics of the recombinant yeast cells transformed with human A1 receptor subtype were investigated with subtype selective adenosine ligands (XAC, DPCPX; CGS-15943; CDS-046142; CDS-046123; NECA,

(R)-PIA; IB-MECA and Alloxazine). That competed with [^{3}H] DPCPX in the expecte rank order. Displacement curves recorded with these compounds show the typical steepness with all the ligands, and the data for each of the ligands could be modeled by one-site fit. The apparent dissociation constants estimated for the individual compounc from the curves (Table 5) are consistent with value published for the receptor obtained from other sources.

Table 5

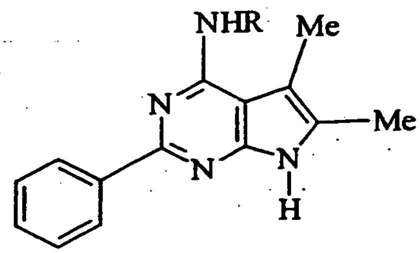
Ki values for membranes from yeast cells transformed with human A1 receptor subtyp

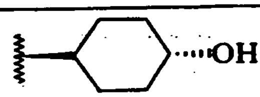
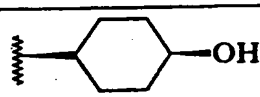
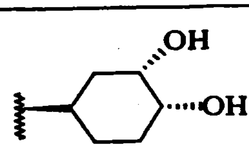
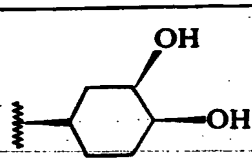
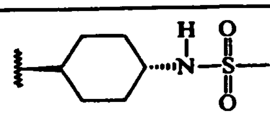
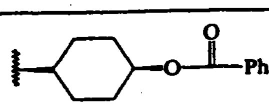
Ligands	K_I (nM)
XAC	5.5
DPCPX	7.1
CGS-1594	10.8
NECA	179.6
(R)-PIA	56.3
IB-MECA	606.5
Alloxazine	894.1
CDS-046142	13.9
CDS-046123	9.8

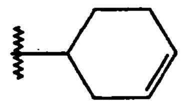
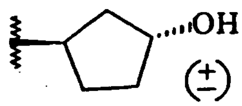
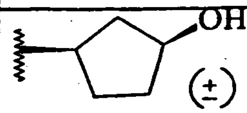
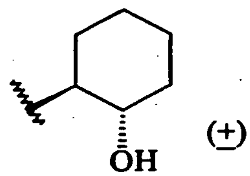
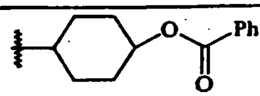
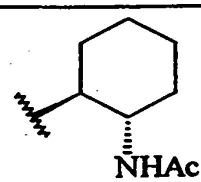
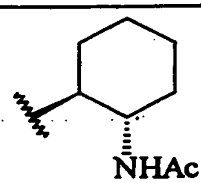
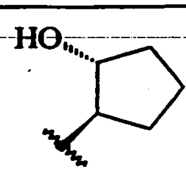
Tables 6 through 12 demonstrate the efficacy and structure activity profiles of deazapurines of the invention. Tables 13 and 14 demonstrate selectivity can be achieve for human adenosine receptor sites by modulation of the functionality about the deazapurine structure. Table 14 also demonstrates the surprising discovery that the compounds set forth therein have subnanomolar activity and higher selectivity for the A_{2b} receptor as compared to the compounds in Table 13.

TABLE 6

Activity of CDS-046142 Series: Effect of N_6-Substituent



		A1	
Code	R	Binding Ki (nM)	Yeast IC50 (nM)
CDS-046142		13.9	97.2
CDS-062365		1423	>10,000
CDS-069533		483.5	>10,000
CDS-069534		196.6	4442.0
CDS-056176		>10,000	>10000
CDS-056175		>10000	>10000
CDS-062352		297.9	>10000

CDS-062351		309.7	>10000
CDS-090909		29.1	
CDS-090910		193.9	
CDS-090913		411.5	
CDS-062352		785.6	>10000
CDS-092474	 Trans (S,S)	64.8	
CDS-092475	 Trans (R,R)	6726.0	
CDS-091175	 (dl)	32.1	

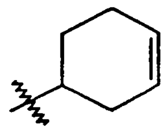
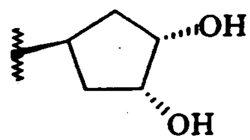
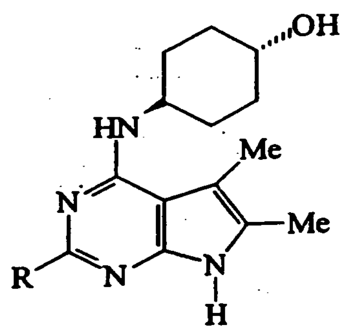
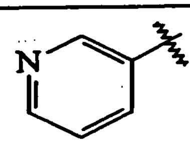
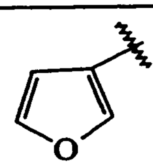
CDS-062351	 (dl)	816.9	2577.0
CDS-090914		34.3	

TABLE 7

Activity of CDS-046142 Series: Effect of C_2-Substituent



		A1	
Code	R	Binding Ki (nM)	Yeast IC50 (nM)
CDS-069532		604.5	>10000
CDS-090895		157.7	763.1

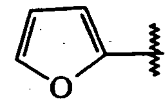
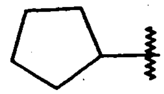
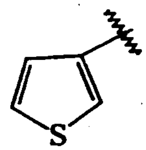
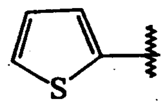
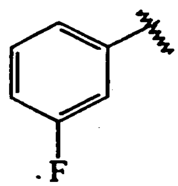
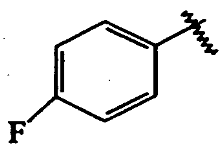
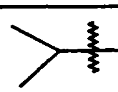
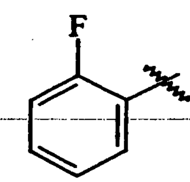
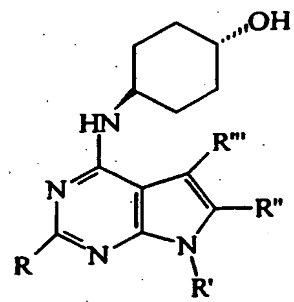
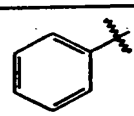
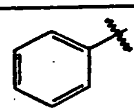
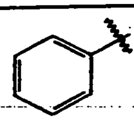
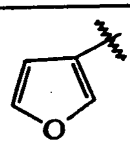
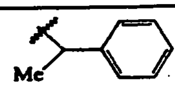
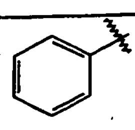
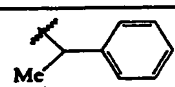
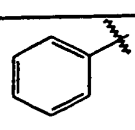
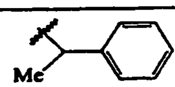
CDS-065564		198.5	2782.5
CDS-090896		443.6	>10000
CDS-090903		61.1	297.0
CDS-090890		30.1	194.7
CDS-090915		19.9	
CDS-090912		62.8	
CDS-090936		2145	
CDS-090177		48.7	

TABLE 8

Activity of CDS-046142 Series: Effect of Pyrrole Ring Substituent



					A1	
Code	R	R'	R"	R'"	Binding Ki (nM)	Yeast IC50 (nM)
CDS-078187		Me	Me	Me	3311	>10000
CDS-090905		H	Me	H	22.3	148.3
CDS-090921		H	H	Me	8.9	
CDS-090902	 O	 Me	Me	Me	2210	>10000
CDS-056090		 Me	Me	Me	863.1	
CDS-056091		 Me	Me	Me	4512	

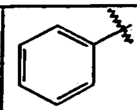
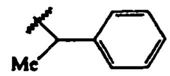
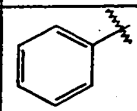
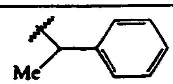
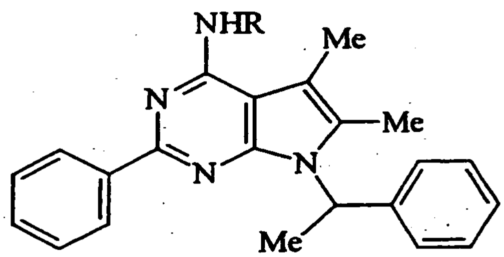
CDS-056089			Me	Me	8451	
CDS-056092			Me	Me	35.3	

TABLE 9



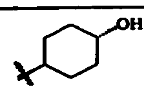
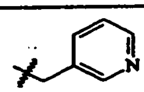
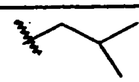
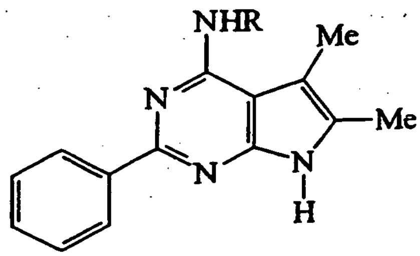
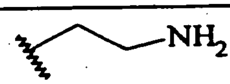
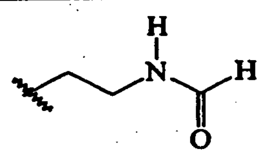
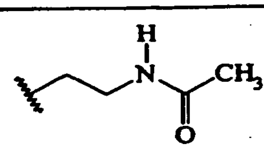
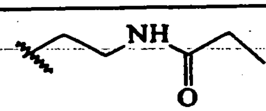
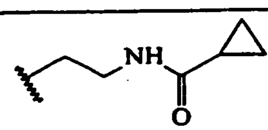
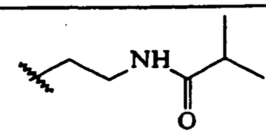
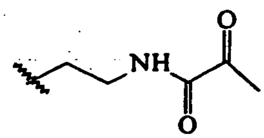
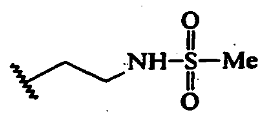
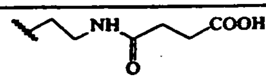
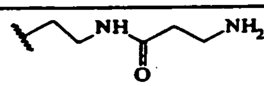
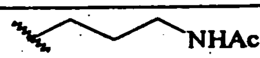
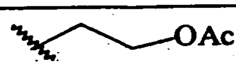
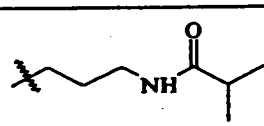
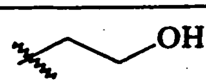
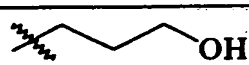
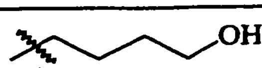
		A1	
Code	R	Binding Ki (nM)	Yeast IC50 (nM)
CDS-056090		863.1	
CDS-056091		4512	
CDS-056089		8451	
CDS-056092		35.3	

TABLE 10

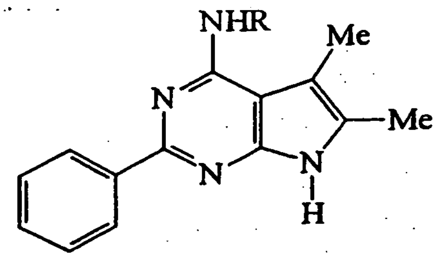
Activity of CDS-046123 Series: Effect of N_6-Substituent

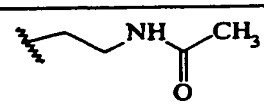
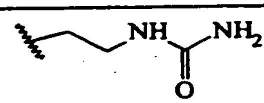
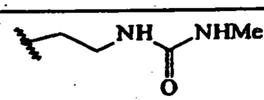
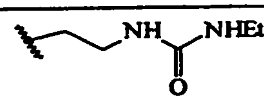
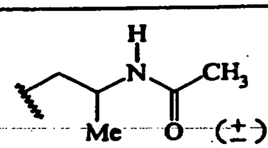
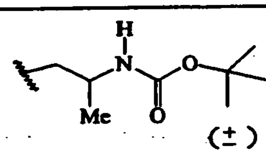


		A1	
Code	R	Binding Ki (nM)	Yeast IC50 (nM)
CDS-062354		1789	>10000
CDS-067146		54.4	1865
CDS-046123		9.8	82.8
CDS-062357		26.7	195.7
CDS-062355		32.8	545.8

CDS-062356		147.5	3972
CDS-067325		151.7	2918
CDS-062392		692.5	>10000
CDS-062393		93.1	3217
CDS-062394		475.3	>10000
CDS-067227		674.9	9376.0
CDS-065568		121.9	2067.5
CDS-066956		233.9	3462
CDS-067038		270.1	3009.5
CDS-062358		384.9	2005
CDS-062359		179.3	3712
CDS-062360		176.1	5054

Activity of CDS-046123 Series: Effect of N_6-Substituent



		A1	
Code	R	Binding Ki (nM)	Yeast IC50 (nM)
CDS-046123		9.8	115.4
CDS-069535		53.9	551.0
CDS-090894		10.3	101.3
CDS-062301		71.1	3217
CDS-090904		6.5	58.7
CDS-090906		105.4	472.1

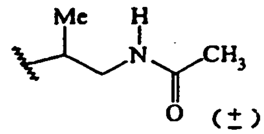
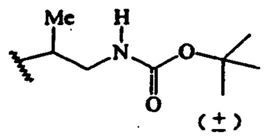
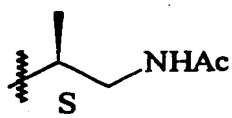
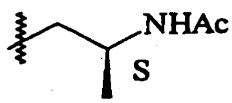
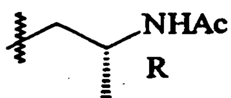
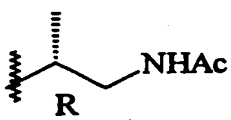
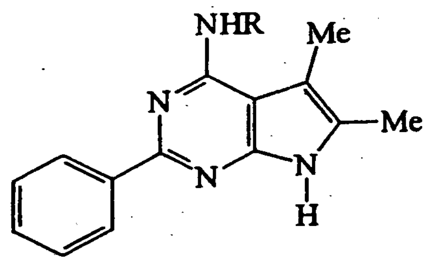
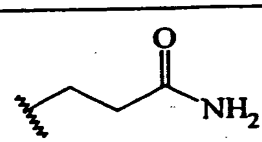
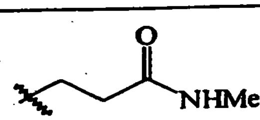
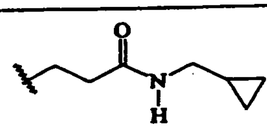
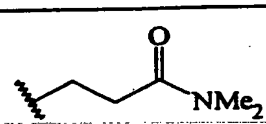
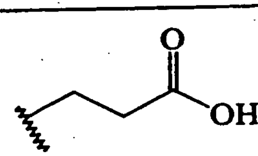
CDS-090908		27.8	162.4
CDS-090907		126.5	1297.0
CDS-092473		2.3	
CDS-095450		9.0	
CDS-095451		17.3	
CDS-091183		2.5	
CDS-091184		213	

TABLE 12

"Retro-Amide" Analogues of CDS-046123



		A1	
Code	R	Binding Ki (nM)	Yeast IC50 (nM)
CDS-065567		16.5	189.4
CDS-090891		7.4	45.7
CDS-062373		95.8	3345.0
CDS-090893		529.1	4040.0
CDS-062371		1060.0	>10000

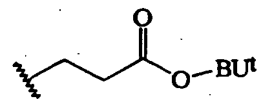
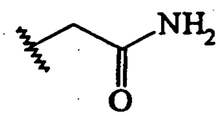
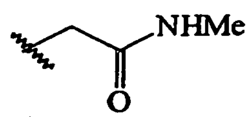
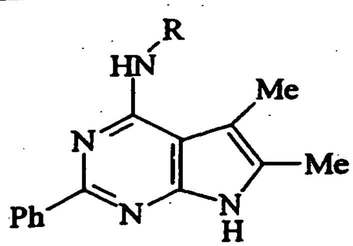
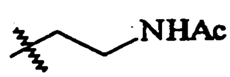
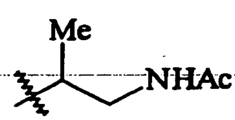
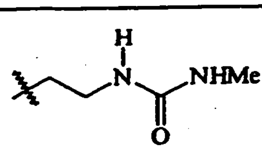
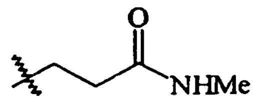
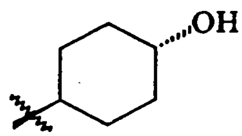
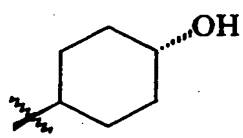
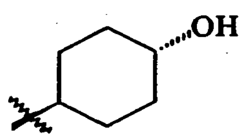
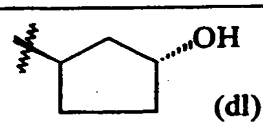
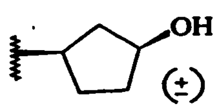
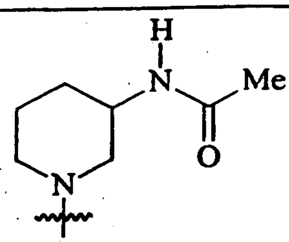
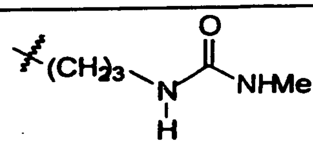
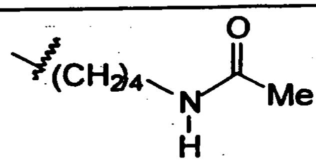
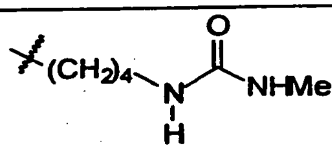
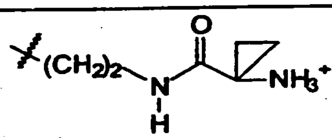
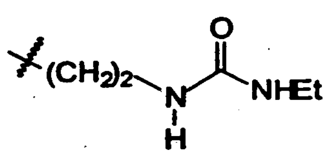
CDS-062372		1272	>10000
CDS-065566		50.8	4028
CDS-065565		48.5	701.5

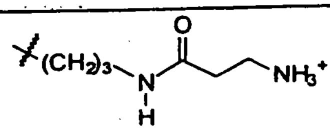
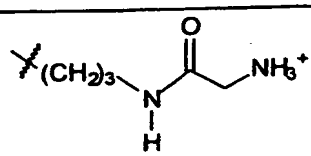
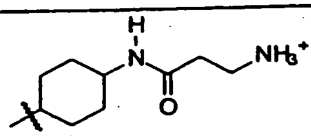
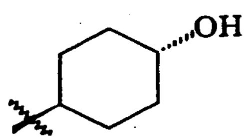
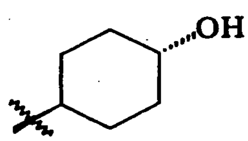
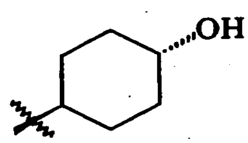
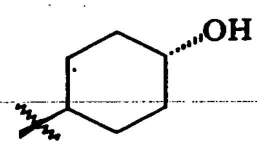
TABLE 13

Profile of Selective Adenosine Antagonists

		Binding Ki (nM)			
	R	A1	A2a	A2b	A3
CDS-046123		9.8-25.1	18.0-48.6	80.3	513.0
CDS-090908		27.8	50.7	84.6	429.8
CDS-090894		20.2	75.6	20.1	4.3

CDS-090891		17.4	111.3	120.6	44.6
CDS-046142		13.9-30.9	933.7	138.0	21.5
CDS-090890[1]		46.6	730.9	30%	9.9
CDS-090905[2]		16.4	766.3	168.3	71.7
CDS-090909		29.1	190.6	1143.0	3.1
CDS-90910		180	230	670	1.0

CDS-116676		40	109	109	0.3
CDS-121180		255	76%	275	≤2.6
CDS-121178		531	981	736	5.3
CDS-121179		443	2965	375	≤6.2
CDS-123264[3]		30%	65%	515	24
CDS-062391		87	204	30	0.02

CDS-121181		75,000	720,000	3,400	507
CDS-121268		333	710,000	710,000	97
CDS-121272		710,000	710,000	720,000	369
CDS-096370[4]		3.7±0.5	630± 56.4	2307± 926	630±76
CDS-113760[4,5]		1.8	206	802	270
CDS-116665[4,6]		8.0	531	530	419
CDS-131921[4,7]		8.0	131	1031	54%[8]

[1]2-thienyl-2-yl; [2]C_5-H; [3] water soluble; [4] R_5 and R_6 are hydrogen; [5] R_3 is 3-fluorophenyl; [6] R_3 is 3-chlorophenyl;[7] R_3 is 4-pyridyl; [8] % activity @ 10 μM

Table 14:

Profile of Selective A_{2b} Antagonists

Code	XR_1	R_2	Binding Data K_i (nM)			
			A_1	A_{2a}	A_{2B}	A_3
CDS-129851	-O-Ph	Me	41.7	21	0.3	14.6
CDS-143995	-O-Ph(p)F	Me	33	58	0.01	18
CDS-143994	-O-Ph(p)Cl	Me	825	591	0.3	60
CDS-143988	-N-pyridin-2-one	Me	60	41	47	48
CDS-143996	-NH-Ph	Me	49	31	109	57

Incorporation by Reference

All patents, published patent applications and other references disclosed herein are hereby expressly incorporated herein by reference

Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.